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THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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PROCEEDINGS OF THE FORTY-THIRD ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1927.

The forty-third annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 31-November 2, 1927.

The meeting was called to order by the president, W. H. MacIntire, Knoxville, Tenn., on the morning of October 31st, at 10 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER, 1928.

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W. H. MACINTIRE, Knoxville, Tenn.

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G. G. FRARY.

Recommendations of Referees.]

(Figures in parentheses refer to year in which appointment expires.)

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SUBCOMMITTEE A: J. W. Kellogg (1928), (Department of Agriculture, Harrisburg, Pa.), *Chairman*; A. G. McCall (1930); R. N. Brackett (1932). [Waters, brine, and salt; tanning materials and leathers; insecticides and fungicides (fluorine compounds); caustic poisons; soils and liming materials (reaction value of soils, liming materials, less common metals in soils); feeding stuffs (stock feed adulteration, mineral mixed feeds, determination of moisture); sugars and sugar products (honey, maple products, starch conversion products; drying, densimetric, and refractometric methods; polariscopic methods; chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, nitrogen activity methods in fertilizers, potash); plants (preparation of plant material for analysis, less common metals in plants, total chlorine in plants); paints, paint materials, and varnishes.]

SUBCOMMITTEE B: A. G. Murray (1928), (Food, Drug and Insecticide Administration, Washington, D. C.), *Chairman*; L. E. Warren (1930); H. C. Lythgoe (1932). [Specific gravity and alcohol, spices and other condiments, naval stores (turpentine); drugs (alcohol in drugs, chloroform and carbon tetrachloride, crude drugs, ipecac alkaloids, radioactivity in drugs and water, laxatives and bitter tonics, mercurials, microchemical methods for alkaloids, terpin hydrate, santonin, ether, bioassay of drugs, fluidextract of ginger, ephedra, pilocarpine in tablets, thymol, menthol, bromide-chlorides, oil of chenopodium, sabadilla assay); beers, wines, and distilled liquors.]

SUBCOMMITTEE C: C. F. Whitney (1928), (Laboratory of Hygiene, Burlington, Vt.), *Chairman*; H. A. Lepper (1930); J. O. Clarke (1932). [Dairy products (butter, cheese, malted milk, dried milk, ice cream, milk proteins, qualitative tests); fats and oils; baking powders and baking chemicals; eggs and egg products (total solids, fat, lipoids, and lipid P_2O_5 ; detection of decomposition; water-soluble protein, unsaponifiable matter, and ash); food preservatives, coloring matters in foods, metals in foods, fruits and fruit products (ash in fruit products, fruit acids), canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products (separation of meat proteins), gelatin, cacao products (crude fiber, cacao butter), cereal foods (sampling of flour, ash in flour and gasoline color value, glutenin in flour, hydrogen-ion concentration of flour, diastatic value of flour, starch in flour, flour-bleaching chemicals, methods for bread analysis—(a) sampling and determination of moisture, (b) lipoids and fat in bread, (c) milk solids in milk bread, (d) rye flour in rye bread—experimental baking tests, moisture in alimentary pastes, unsaponifiable matter in flour and in alimentary pastes and water-soluble protein in alimentary pastes).]

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General referee: C. H. Badger, Food, Drug and Insecticide Adm., Washington, D. C.

TANNING MATERIALS AND LEATHERS:

General referee: I. D. Clarke, Bureau of Chemistry and Soils, Washington, D. C.

INSECTICIDES AND FUNGICIDES:

General referee: J. J. T. Graham, Food, Drug and Insecticide Adm., Washington, D. C.

FLUORINE COMPOUNDS:

Associate referee: G. A. Shuey, Agricultural Experiment Station, Knoxville, Tenn.

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General referee: C. M. Smith, Food, Drug and Insecticide Adm., Washington, D. C.

SOILS AND LIMING MATERIALS:

General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

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a. ALKALINE SOILS:

Associate referee: P. S. Burgess, Agricultural Experiment Station, Tucson, Ariz.

b. ACID SOILS:

Associate referee: E. T. Wherry, Bureau of Chemistry and Soils, Washington, D. C.

LIMING MATERIALS:

Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS:

Associate referee: J. S. McHargue, Agricultural Experimental Station, Lexington, Ky.

FEEDING STUFFS:

General referee: W. F. Sterling, Food, Drug and Insecticide Adm., Washington D. C.

STOCK FEED ADULTERATION:

Associate referee: H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

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NITROGEN ACTIVITY METHODS IN FERTILIZERS:

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POTASH:

Associate referee: L. D. Haigh, Agricultural Experiment Station, Columbia, Mo.

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Associate referee: H. R. Kraybill, Agricultural Experiment Station, Purdue, Ind.

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Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

TOTAL CHLORINE IN PLANTS:

Associate referee: Doris Tilden, Food, Drug and Insecticide Adm., San Francisco, Calif.

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General referee: W. F. Hand, Agricultural and Mechanical College, Miss.

SPECIFIC GRAVITY AND ALCOHOL:

General referee: A. W. Hanson, Food, Drug and Insecticide Adm., Minneapolis, Minn.

SPICES AND OTHER CONDIMENTS:

General referee: W. C. Geagley, Food and Drug Department, Lansing, Mich.

NAVAL STORES:

General referee: F. P. Veitch, Bureau of Chemistry and Soils, Washington, D. C.

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Associate referee: V. E. Grotlisch, Food, Drug and Insecticide Adm., Washington, D. C.

DRUGS:

General referee: A. E. Paul, 1625 Transportation Bldg., Chicago, Ill.

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Associate referee: E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

CHLOROFORM AND CARBON TETRACHLORIDE:

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Associate referee: J. W. Sale, Food, Drug and Insecticide Adm., Washington, D. C.

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Associate referee: C. K. Glycart, Food, Drug and Insecticide Adm., Chicago, Ill.

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Associate referee: C. W. Harrison, Food, Drug and Insecticide Adm., Baltimore, Md.

SANTONIN:

Associate referee:

ETHER:

Associate referee: H. M. Joslin, Bureau of Chemistry and Soils, Washington, D. C.

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Associate referee:

FLUID EXTRACT OF GINGER:

Associate referee: J. F. Clevenger, Food, Drug and Insecticide Adm., Washington, D. C.

EPHEDRA:

Associate referees: C. K. Glycart and A. E. Paul, Food, Drug and Insecticide Adm., Chicago, Ill.

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Associate referee: T. F. Pappe, Food, Drug and Insecticide Adm., Baltimore, Md.

THYMOL:

Associate referee: F. L. Hart, Food, Drug and Insecticide Adm., Chicago, Ill.

MENTHOL:

Associate referee: F. L. Elliott, Food, Drug and Insecticide Adm., Baltimore, Md.

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OIL OF CHENOPODIUM:

Associate referee: E. K. Nelson, Bureau of Chemistry and Soils, Washington, D. C.

SABADILLA ASSAY:

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DAIRY PRODUCTS:

General referee: H. C. Lythgoe, Department of Public Health, Boston, Mass.

BUTTER:

Associate referee: L. C. Mitchell, 204 Old Custom House, St. Louis, Mo.

CHEESE:

Associate referee: E. O. Huebner, Dairy and Food Commission, Madison, Wis.

MALTED MILK:

Associate referee: B. G. Hartmann, Food, Drug and Insecticide Adm., Washington, D. C.

DRIED MILK:

Associate referee: J. T. Keister, Food, Drug and Insecticide Adm., Washington, D. C.

ICE CREAM:

Associate referee: R. O. Baird, Food and Drug Laboratory, Bismarck, N. Dak.

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BAKING POWDERS AND BAKING CHEMICALS:

General referee: L. H. Bailey, Food, Drug and Insecticide Adm., Washington, D. C.

EGGS AND EGG PRODUCTS:

General referee: J. C. Palmer, Food, Drug and Insecticide Adm., Seattle, Wash.

TOTAL SOLIDS, FAT, LIPOIDS, LIPOID P_2O_5 :

Associate referee: J. C. Palmer, Food, Drug and Insecticide Adm., Seattle, Wash.

DETECTION OF DECOMPOSITION:

Associate referee: H. I. Macomber, Food, Drug and Insecticide Adm., New York City.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, AND ASH:

Associate referee: Samuel Alfend, Food, Drug and Insecticide Adm., St. Louis, Mo.

FOOD PRESERVATIVES:

General referee: W. W. Randall, State Department of Health, Baltimore, Md.

COLORING MATTERS IN FOODS:

General referee: C. F. Jablonski, Food, Drug and Insecticide Adm., New York City.

METALS IN FOODS:

General referee: W. F. Clarke, Bureau of Chemistry and Soils, Washington, D. C.

FRUITS AND FRUIT PRODUCTS:

General referee: H. J. Wichmann, Food, Drug and Insecticide Adm., San Francisco, Calif.

ASH IN FRUIT PRODUCTS:

Associate referee: Doris Tilden, Food, Drug and Insecticide Adm., San Francisco, Calif.

FRUIT ACIDS:

Associate referee: B. G. Hartmann, Food, Drug and Insecticide Adm., Washington, D. C.

CANNED FOODS:

General referee: V. B. Bonney, Food, Drug and Insecticide Adm., Washington, D. C.

VINEGARS:

General referee: J. O. Clarke, Food, Drug and Insecticide Adm., Washington, D. C.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

General referee: J. W. Sale, Food, Drug and Insecticide Adm., Washington, D. C.

MEAT AND MEAT PRODUCTS:

General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

SEPARATION OF MEAT PROTEINS:

Associate referee: W. S. Ritchie, University of Missouri, Columbia, Mo.

GELATIN:

General referee: E. H. Berry, Food, Drug and Insecticide Adm., Chicago, Ill.

CACAO PRODUCTS:

General referee: H. A. Lepper, Food, Drug and Insecticide Adm., Washington, D. C.

CRUDE FIBER:

Associate referee: Marie L. Offutt, Food, Drug and Insecticide Adm., New York City.

CACAO BUTTER:

Associate referee: W. F. Baughman, Bureau of Chemistry and Soils, Washington, D. C.

CEREAL FOODS:

General referee: F. C. Blanck, Bureau of Chemistry and Soils, Washington, D. C.

SAMPLING OF FLOUR:

Associate referee: H. Runkel, Food, Drug and Insecticide Adm., Minneapolis, Minn.

ASH IN FLOUR AND GASOLINE COLOR VALUE:

Associate referee: D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C.

GLUTENIN IN FLOUR:

Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

HYDROGEN-ION CONCENTRATION OF FLOUR:

Associate referee: C. H. Bailey, University of Minnesota, Minneapolis, Minn.

DIASTATIC VALUE OF FLOUR:

Associate referee: E. L. Tague, State Agricultural College, Manhattan, Kans.

STARCH IN FLOUR:

Associate referee: L. H. Bailey, Food, Drug and Insecticide Adm., Washington, D. C.

FLOUR-BLEACHING CHEMICALS:

Associate referee: G. C. Spencer, Bureau of Chemistry and Soils, Washington, D. C.

METHODS FOR BREAD ANALYSIS:**a. SAMPLING AND DETERMINATION OF MOISTURE:**

Associate referee: L. H. Bailey, Food, Drug and Insecticide Adm., Washington, D. C.

b. LIPOIDS AND FAT IN BREAD:

Associate referee: Samuel Alfend, 204 Old Custom House, St. Louis, Mo.

c. MILK SOLIDS IN MILK BREAD:

Associate referee: L. H. Bailey, Food, Drug and Insecticide Adm., Washington, D. C.

d. RYE FLOUR IN RYE BREAD:

Associate referee:

EXPERIMENTAL BAKING TESTS:

Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

MOISTURE IN ALIMENTARY PASTES:

Associate referee: S. C. Rowe, Food, Drug and Insecticide Adm., Washington, D. C.

UNSAPOⁿIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES AND WATER-SOLUBLE PROTEIN IN ALIMENTARY PASTES:

Associate referee: Samuel Alfend, 204 Old Custom House, St. Louis, Mo.

BEERS, WINES AND DISTILLED LIQUORS:

General referee: W. V. Linder, Bureau of Internal Revenue, Washington, D. C.

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PRESIDENT'S ADDRESS.¹

THE RÔLE OF THE LYSIMETER IN SOIL CHEMISTRY RESEARCH.

By W. H. MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.).

It is most interesting and instructive to study the history of this association through the reading of presidential addresses in their chronological order. One is impressed with the progressive development in the scope of the association's activities, and the ready adaptation of its machinery to new conditions and requirements. One is also impressed by the fact that the list of those who have served this association constitutes a roster of those who have been in a large measure responsible for the development of the science of chemistry in its relation to American agriculture. One finds also that for some time after the founding of this association the interests of the more active participants in its workings were similar. As a consequence, most of the annual addresses were either of a general philosophical character or in the nature of recommendations concerning the problems, responsibilities, and possibilities peculiar to the association. Such intensive and continuous thought by so many constructive minds resulted in a comprehensive survey and digest of the functions of the organization and of the manner in which those functions should be discharged. Little remains to be pointed out along those lines by us of the present day.

As one advances in the reading of the annual proceedings, and parallels such reading by noting the development of the *Book of Methods*, two points stand out. *First*, there was a steady increase in the scope of commodities that were made subject to regulation, and so recognized by the association, with either the attendant or the casual factor, that of the development of special fields by many of its members. *Secondly*, there developed a positive recognition of the necessity for research in the verification and perfection of old methods and the origination of new procedures. These two factors are reflected in the personnel of the present membership roster, and in the representative character of the large registration of those in attendance at the annual sessions. There is evidence of a sympathetic attitude on the part of the diversified chemical industries that relate to agriculture and allied interests. There is also evidence of the growing appeal to, and the collaboration by, those interested primarily in research. It follows that different individuals among the mem-

¹ Presented Tuesday morning, November 1st, as special order of business for 11 o'clock.

bership will be looked upon as representatives of their special lines of endeavor. Likewise, it follows that such representatives should feel an obligation to bring something of value and interest to the membership as a whole, by the perfection of needed methods or by the presentation of timely papers. Accordingly, it seems that an address by your President might well be a contribution from the group that he represents. In a few instances this has been the case. It would seem to be especially desirable that such a custom be fostered, now that in the order of things it may not be expected that any one specialty will often be recognized by the attainment of one of its proponents to the presidency. With this



1. OUTSIDE VIEW OF PIONEER PIT LYSIMETER INSTALLATION.

thought in mind, the present treatise is offered as a brief and inadequate contribution from that important and rapidly expanding branch of research—soil chemistry.

During the past forty years this field has passed through a remarkable transition, characterized by rectification and clarification as to concept and teaching, and by the inauguration of many new lines of attack. There occurred a distinct trend from the old static idea to the present dynamic concept of soils as a complex system. Concurrently, there came a marked advance in the development of analytical methods—chemical, micro-chemical, biochemical, and petrographic—as tools of attack. The rise of the science of bacteriology, with its explanation of biochemical generation of nutrient salts, and that of plant physiology, with its explanations as to assimilation of nutrients and photosynthetic elaboration; the development of organic chemistry; and the advent of colloid chemistry have

had a most important influence upon the usefulness of chemistry in its relation to soils.

Recognition of the importance of conserving our chief national asset—soil fertility—has resulted in increased State and Federal support, so that there has been a steady increase in the number of individuals engaged solely in soil chemistry research. Improved curricula and greater opportunities for graduate training have qualified and inspired a larger number of workers. The problems to be solved have become more and more varied and of greater magnitude. The advent of irrigation in arid and semi-arid regions, in particular, has been followed by problems of deep academic interest and tremendous practical importance. Specialization within the field of soil chemistry has, therefore, been the logical and inevitable result. Many of the problems now under investigation, and even restricted phases of such problems, are so important and intricate as to call for one's life work.

The fertility of a soil is measured by what it imparts to the plant. Studies as to the ability of a soil to support plant growth may be approached from different angles. In this country considerable progress has been made by means of the lysimeter—from the Greek *lysos*, a loosening, plus *meter*. One or more installations of this type of equipment have been made in the Florida, Hawaii (S. P.), Cornell, New York, Oregon, Missouri, Virginia, Illinois, North Carolina, and Tennessee Experiment Stations. One who is highly esteemed in the councils of agricultural research has stated that "A station is hardly to be considered complete without a lysimeter equipment". A lysimeter installation may be defined as an outdoor adjunct laboratory for the housing of equipment to provide material for study in the chemical laboratory. Each unit, tank, or container is embedded in the soil and provided with a suitable filter bed and drainage outlet to convey rainfall percolations to receptacles placed in a structure which insures proper temperature control and comfort for the workers. Reinforced concrete, galvanized iron, and terra cotta containers have been used, generally with block tin outlets. In any case it is essential that the walls of the container shall be resistant to the action of salt- and gas-impregnated soil waters.

The layman often holds an erroneous idea as to the real objectives for which the lysimeter is intended. He thinks of it as a means of determining the exact total of plant nutrients that an acre of soil may lose. It is feasible to establish certain differences that may serve as a guide or basis for fundamental agronomic studies, even though the nutrient content of percolates may not give a measure that can be translated, without qualification, into the economics of field practice. But whatever value this rôle may have, it is considered as only incidental by the soil chemist, who imposes different conditions, observes variations, and then seeks the

explanation for the phenomena indicated by the several concentrations of solutes in the percolates.

A lysimeter equipment may be used to study the gaseous phases in soil and subsoil atmospheres, to measure the formation of readily removable combinations, or else to indicate the formation of combinations which resist the leaching action of the free soil water. There are, of course, systems which are retained against normal percolation, so tenaciously are they held by the soil complex through the medium of the interface condensates upon the soil particles—systems which may nevertheless yield to the intensive pull of foraging root hairs. Still there exists a ten-



2 INSIDE VIEW OF PIONEER PIT LYSIMETER INSTALLATION

dency toward attainment of equilibria between the surface-moisture layers and the gravitational water of a soil system. Indeed, there is some evidence of a close parallel between certain components of a displaced solution and those obtained in the first percolates subsequent to prolonged periods of aridity. The dynamic conditions inherent in the soil are influenced, of course, by the seasonal bacterial activities, and such variations may be advantageously studied by the periodic collection of percolates.

There are two views regarding the proper manipulation of a soil to be used in lysimeter tanks. One school advocates that the container be built to enclose a selected block of soil *in situ*. It is contended that the disruption and mixing of a body of soil may cause an alteration of the state of its colloid content, and that the coincident aeration may also be responsible for an abnormality in the activities of the soil flora—an ab-

normality which might persist for a number of years. The force of this contention—yet to be substantiated—is greatly minimized if we assume that precautions will be taken to limit exposure and prevent the drying of the soil during handling, so that the placed soil mass may be considered as having undergone a mechanical disruption no more violent than that which would result from a plowing, discing, and harrowing under direct exposure to the sun's rays. A counter argument is that when several closely adjacent areas are chosen for enclosure *in situ* the assumed homogeneity of the soil may sometime be disproved only after the termination of a project with belated knowledge of wasted effort. Furthermore, this method would tend to limit the number of units in an installation, in view of the fact that any lack of uniformity would increase with the size of the area intended for enclosure. This method is also very expensive.

The second school holds that the assurance of homogeneity of the soil mass should be the first essential. The abnormal aeration incident to thorough mixing, with proper protective measures, is considered as being a uniform experimental factor, and one that tends toward an early return to normalcy. This method admits of the use of a relatively large mass of homogeneous soil, and a large number of units for a multiplicity of additive treatments and sufficient replications.

One outstanding essential to the successful and adequate study of the composition of soil percolates, and especially if absorption, interchange, and repression phenomena are to be studied, is the placement of a soil with and without an underlying stratum, or strata, of subsoil. By such a parallel it has been possible to establish, in comparatively short periods of time, certain principles and relationships which otherwise would have been determined only after protracted periods, if at all. The influence which a growing crop exerts upon the soil system may also be studied advantageously by the use of lysimeters of different depths.

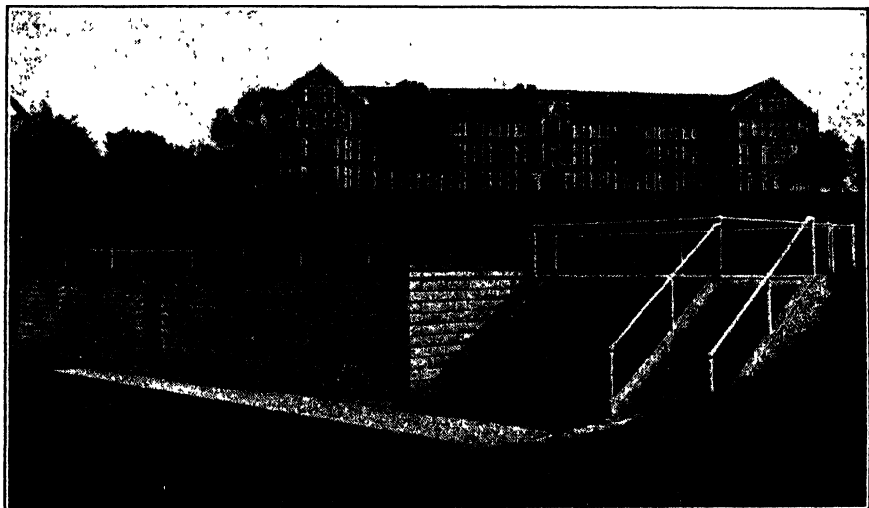
It is true that many fundamental studies may be pursued fruitfully by soil cultures, and that direct measurement of induced changes may be made by a study of either the soil complex or its extraction or replacement solutions. Such studies have their place and value. Nevertheless, the chief factor having to do with the inherent fertility of a soil and the maintenance of such fertility is the outgo induced by rainfall—it being realized, of course, that the present discussion is restricted to actual percolation and does not consider the losses involved through surface erosion.

In the forcing of an extraction of the soil mass there is always the deterrent factor of buffer effect and the inadequacy and limitations of methods under the vitiating influence of some component of the complex systems. For example, the recovery of sulfates by a hydrochloric acid extraction is nearly always less than that obtained through an extraction with distilled water. The clear percolate, however, may be analyzed by

means of precise and reliable methods, such as those that are followed in the analysis of waters.

The lysimeter has established a number of fundamental principles, some of which prove the fallacies of deductions made from data obtained under conditions imposed in the laboratory. Moreover, the way has been pointed to new methods of approach to problems which may be attacked advantageously from the synthetic viewpoint, and with media of known initial composition. A few findings may be pointed out.

Uncertainties as to the influence of depth and character of subsoil upon the speed of percolation and the extent to which rainfall is conserved have



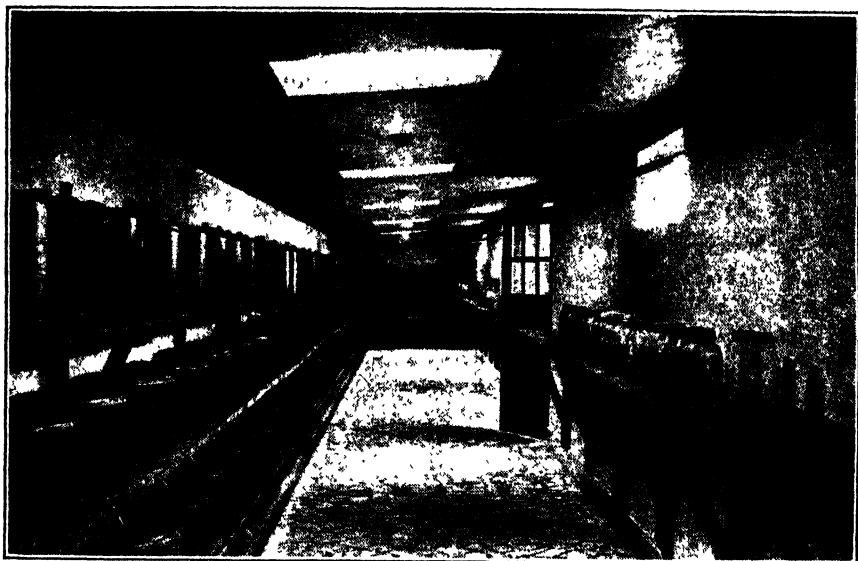
3. OUTSIDE VIEW OF HILLSIDE OR TERRACE TYPE OF LYSIMETER INSTALLATION.

been clarified. The relationships between nitrogen losses from fallow, legume cropping, and non-legume cropping have been established. The speed and extent of the outgo of added nitrogen have been shown to be influenced in marked degree by the type and depth of subsoil. The influence of the cation effect upon the rapidity of the downward movement of nitrogen through the subsoil has been demonstrated. It has also been shown that the surface run-off waters, when such are free of suspended material, may be practically devoid of plant nutrients. The influence of ameliorant incorporations upon the balance between increment and outgo of anions and cations has been shown. The absence of basic interchange in the *surface soil* and the uniform and antithetic "reciprocal repression" induced by different forms and quantities of lime or magnesia have been demonstrated for the heavier types of soil under humid conditions. Conversely, the opposite effect of base-interchange has been established as prevailing in the more hydrated subsoils of humid regions.

By "reciprocal repression" is meant the protective effect that is evidenced by marked decrease in calcium outgo as a result of magnesian additions and the similar decrease in magnesium outgo which follows the use of high-calcic lime. This latter finding probably explains, in part, at least, the deleterious effect produced by lime upon such magnesia-loving plants as tobacco. Again, it has been shown that either lime or magnesia added in any form or quantity produces a marked decrease in the solubility of the native potassic components. It has been demonstrated that such a repression of solubility obtains even in the presence of large quantities of neutral calcium or magnesium salts, and after all of either a caustic or a carbonate ameliorant has been "fixed" by the soil mass. This is diametrically opposed to the deductive teaching that a liberation of the insoluble potash of the soil will follow the use of lime. Furthermore, correlated studies have shown that additions of the lime or magnesia cause a decided decrease in the loss of simultaneously added soluble potassic materials, with the assumption—yet to be proved—that such retained additions may be, nevertheless, available to growing plants.

Likewise, it has been shown that where additions were above certain limits the outgo of carbonate liming materials was not governed by the amount of the incorporation, or by the residual carbonate content, but rather by the concentration of carbon dioxide in the soil solution. It has also been shown that there is a marked difference between the early and subsequent solubility of fixation products derived from additions of calcium or magnesium. The maximum annual outgo of added and absorbed lime or magnesia during the initial year may be accounted for in part by the marked acceleration in oxidative processes during the first year and the decided drop in outgo thereafter may be attributed to a reversion to normal biochemical activities. But the marked difference between the annual ratio of outgo to absorbed material for successive years demonstrated that the absorption complexes become more and more resistant to hydrolysis. The absorbed materials apparently acquire properties similar to those of the native non-carbonate calcic and magnesian complexes. Furthermore, the enhanced outgo of calcium or magnesium constituted such a small fraction of the addition that a need for reliming must be attributed in part to the acquired state of the absorbed residue, rather than solely to its depletion through leaching. From this there came a further study as to the advisability of first decreasing the acidity of a soil and then insuring fresh streams of lime or magnesia, or both, through repetition of small incorporations to care for the factors of decreasing solubility and replacement of the losses induced by percolation and through assimilation by the growing plant. Again, the speed of the carbonation and absorption of economic quantities of burnt and hydrated limes, and the persistence of larger quantities in the caustic form, have been determined. Considerable has been learned as

to the influence which form, fineness, and type of incorporated materials exert upon their fixation and outgo. Likewise, it has been determined that the depth to which an incorporation is made has a marked influence upon the conservation of the added materials and upon the combinations in which the basic elements pass to the subsoil. For example, when an economic incorporation of either of the commercially used types of high-calcic lime was made in the upper 4 inches of the surfaced soil there was a loss of but 6 per cent from the feeding zone of shallow-rooted plants over a four-year period, as against a 31 per cent, or 5-fold, loss when the same incorporations were made in the second 4-inch zone.



4. INSIDE VIEW OF HILLSIDE OR TERRACE TYPE OF LYSIMETER INSTALLATION

Under similar conditions, the balance between additions, enhanced outgo, and residual carbonates showed that the lower-zone incorporations of a coarse limestone "separate" had undergone a disintegration eight times that brought about in the upper zone. As related to this finding, it was also determined that the oxidative processes may be much more extensive in the protected lower zone of the surface soil than in the exposed upper zone.

From an academic viewpoint the lysimeter has proved of value in determining the nature of the processes by which the compounds derived from added materials are retained in the soil. It has been held by some soil chemists that added solutes are retained by the soil solely through absorptive phenomena, and without the formation of solids of definite chemical proportions. Others have contended that absorption is accom-

plished in stoichiometrical relationships. The complexity and indefiniteness of the combinations which make up the soil system have been a serious handicap in attempts to clarify and harmonize these two concepts. Happily, the lysimeter has been of value in pointing a way to helpful studies in this connection.

In some of the later Tennessee studies lime and magnesia were used in calcium oxide equivalents ranging between 250 pounds and 200,000 pounds per two million pounds of soil. It was found that the outgo of sulfates was accelerated by the use of all forms of both calcium and magnesium in moderate quantities, and in the case of magnesian additions the increase in rate was followed by heavy increases in engendered and percolated sulfates. The reverse effect was produced by increases in rates of caustic lime. Complete absence of sulfates in the percolates was noted, even when the normal soil sulfates were augmented by a considerable rainfall increment and by heavy additions of soluble sulfates. Thus, heavy additions of caustic lime rendered the sulfate radical insoluble, either through absorption or through the formation of an insoluble sulfate. In time it was established that the persistence of the sulfate fixation was governed by the maintenance of a certain concentration of hydroxyl ions in the free-soil water. Amplifying laboratory studies showed the same retentive property to be exercised to a still greater extent by the residues of ignited soils. This was followed by studies with the pure gels of the three dominant oxides that go to make up the inorganic soil colloids. From those studies proof was adduced to show the formation of certain binary systems and a ternary system, $3 \text{ CaO} \cdot \text{Al}_2\text{O}_3 \cdot 3 \text{ CaSO}_4 \cdot 33 \text{ H}_2\text{O}$, and its ferric analogue, which had not been found previously. These systems were later isolated from each of several soils and photographed through the microscope. It was accordingly established that the fixation of sulfates, to the extent of several thousand pounds per acre, which had been determined by the lysimeter studies, was due not to physical absorption phenomena but to the formation of definite crystallates. The controlled formation of the calcium-alumino-sulfate may prove of considerable value as a measure of the form and reactivity of colloidal alumina and silica occurrences in soils. In addition to their academic interest to the soil chemist, these findings have been deemed of practical value in the field of ceramics, plaster chemistry, and cement chemistry.

The gravitational movement of organic and inorganic colloids from the A horizon is one subject which has not been studied, and the lysimeter might be adapted to such an investigation. The specific functions of the several colloids in effecting the retention of different nutrient and fertilizing materials might be studied by means of the lysimeter, especially after a pilot investigation with synthetic soils. With the recent development in the methods to determine minute occurrences of phosphates and

to distinguish between organic and inorganic forms in solution, the lysimeter may open the way to studies concerning the outgo of phosphates. Interesting and instructive studies could doubtless be made by the introduction of modifications which would admit of researches in dialysis under outdoor conditions. When utilized further by those seeking various objectives, outdoor percolation apparatus is certain to be found more and more useful. It may be, and should be, emphasized that the lysimeter is not, therefore, merely a device for measuring either the outgo or the corollary retention of various salts or their component ions, but that it has a great usefulness in the indication of phenomena which may serve as the basis of the most intricate laboratory research.

ORDER OF PUBLICATION.

The reports of the committees presented on the last day of the annual meeting will be given at the beginning of the proceedings rather than in their chronological order. This will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD DAY.

WEDNESDAY—MORNING SESSION.

REPORT OF THE REPRESENTATIVES AT THE NATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH.

The sixth annual meeting of the National Conference on Pharmaceutical Research was held in St. Louis, August 20, 1927.

The following delegates were present:

Association of Official Agricultural Chemists:

J. C. Munch, L. E. Warren.

American Association of Colleges of Pharmacy:

Anton Hogstad, Louis Saalbach, M. S. Dunn.

American Chemical Society, Division on Medicinal Chemicals:

R. P. Fishelis.

American Drug Manufacturers Association:

Mortimer Bye, L. W. Rowe.

American Pharmaceutical Association:

H. V. Arny, W. L. Scoville, J. C. Peacock, G. D. Beal.

American Pharmaceutical Manufacturers Association:

F. B. Fisk.

Bureau of Chemistry, U. S. Department of Agriculture:

L. E. Warren, J. F. Clevenger, G. L. Keenan.

Hygienic Laboratory, U. S. Public Health Service:

G. W. McCoy.

National Association of Boards of Pharmacy:

L. L. Walton, H. W. Renter, H. C. Christensen.

National Association of Retail Druggists:

Louis Emanuel, F. H. Swift.

National Formulary Revision Committee:

W. L. Scoville.

National Wholesale Druggists Association:

E. L. Newcomb.

Pharmaceutical Laboratory Conference:

J. C. Krantz, Jr., C. M. Sterling.

Plant Science Seminar:

E. N. Gathercoal, O. M. P. Canis, H. W. Youngken, L. J. Schwarz.

Proprietary Association:

E. F. Kemp, D. E. Combs, J. H. Howe.

U. S. P. Revision Committee:

E. F. Cook, C. H. LaWall, G. M. Beringer, A. G. DuMez.

The following associates were present:

C. L. Cox, L. K. Darbaker, P. H. Dirstein, E. G. Eberle, G. W. Fiero, E. B. Fischer, D. B. R. Johnson, W. C. Jones, E. F. Kelly, H. A. Nelson, S. E. Owen, Bertha DeG. Peacock, P. S. Pittenger, R. H. Raabe, W. F. Rudd, Albert Schneider, A. J. Schwarz, D. H. Spencer, R. E. Terry, E. R. Theis and W. H. Zeigler.

Reports were presented by the chairmen of seven of the ten standing committees of the conference, those reporting being the following:

<i>Committee</i>	<i>Chairman</i>
Manufacture of Galenicals	G. M. Beringer
Standardization of Galenicals	W. L. Scoville
Standardization of Medicinal Chemicals	C. H. LaWall
Sources and Identification of Botanic Drugs	H. W. Youngken
Standardization of Botanic Drugs	E. L. Newcomb
Chemistry of Drug Plants	J. C. Munch for W. O. Emery
Business Research in Pharmacy	Ambrose Hunsberger

Discussion of the report of the committee on committees resulted in authorization of the creation of the three following standing committees:

Committee on History of Pharmacy.

Committee on Pharmacology and Bioassays.

Committee on Physical Chemistry as Applied to Pharmaceutical Problems.

During the afternoon session a discussion of the financial questions raised in the report ensued and the following actions were taken by the Conference:

(1) That there be no retrenchment in carrying on the activities of the conference during 1927-1928.

(2) That the chairman's report that the routine receipts of the conference were only \$240, whereas the necessary expenditures amounted to about \$400, demanded specific emergency action.

(3) That a rising vote of thanks be extended to the modest and generous anonymous friend who saved the activities of the conference during 1926-1927 by a personal contribution of \$100.

(4) That notice be served, as per requirements of the constitution, of a proposed amendment of the constitution at the meeting of 1928, whereby the annual dues be raised from \$20.00 to \$25.00 per organization member.

(5) That the chairman be authorized to endeavor to secure voluntary subscriptions of \$1.00 each from those to whom bulletins of the conference are sent.

(6) That, since the annual organization dues cannot be raised until 1928, the chairman be authorized to send with the bills for 1927-1928 dues a specific request for an extra donation from each dues-paying member organization.

(7) That a committee of five be appointed by the chairman to study the question of expansion of the activities of the National Conference of Pharmaceutical Research and of providing adequate funds for carrying on such increased activities; such committee to report its findings at the 1928 meeting.

(8) The following subjects were then taken up and informally discussed:

- (a) Census of research.
- (b) Research topics.
- (c) Research grants and prizes.
- (d) Research fellowships.

Under the topic of research grants, it was announced that the State Pharmaceutical Associations of Minnesota, Utah, Missouri, Wisconsin, Michigan, and Maryland had established grants available for research in the pharmacy schools of their respective universities.

After informal discussion of a number of research matters, participated in by Messrs. Jones, Scoville, Kelly, DuMez, Dunn, Munch, Berlinger, Johnson, Dirstein, and Zeigler, the following officers were elected for the year 1927-1928:

Chairman, H. V. Arny, of New York.

Vice-Chairman, E. F. Cook, of Philadelphia.

Secretary-Treasurer, J. H. Krantz, Jr., of Baltimore.

The next meeting will be held on the Saturday before the 1928 meeting of the American Pharmaceutical Association.

J. C. MUNCH.

L. E. WARREN.

Approved.

REPORT OF THE COMMITTEE TO REPRESENT THE A. O. A. C. AT THE FIRST INTERNATIONAL CONGRESS OF SOIL SCIENCE.

The First International Congress of Soil Science was opened by President Coolidge in the Assembly Hall of the U. S. Chamber of Commerce in Washington, June 13, 1927, and continued for a period of 10 days. Officially accredited delegates from 40 countries were present, whereas the total attendance was in excess of 1000. In accordance with instructions given at the last annual meeting, a committee of three was present to extend the greetings of this association. This duty was performed by a committee comprising the secretary of the association, Past President C. A. Browne, and the president of the association.

The proceedings were carried out with dignity and expedition, and the papers and discussions registered a high plane of academic attainment. A complete resumé of papers was given through a published volume of abstracts in French, German, and English. The work reported showed

that the development of the science of chemistry in its relation to soils is keeping pace with similar developments in other fields.

W. H. MACINTIRE.

C. A. BROWNE.

W. W. SKINNER.

Approved.

REPORT OF COMMITTEE ON EDITING METHODS OF ANALYSIS.

The work of the Committee on Editing Methods of Analysis during the past year has been the compilation of the changes made at the 1926 meeting of the association. This compilation was published in the February, 1927, issue of *The Journal*.

A similar compilation, covering the changes made at the 1925 meeting, had already been printed¹ in accordance with the resolution presented² to the Executive Committee and approved by that committee at the 1925 meeting³. The changes made at the 1924 meeting have not been printed in this manner although, of course, they are to be found on record in the reports of Sub-committees A, B, and C of the Committee on Recommendations of Referees, as published in the February, 1925, issue of *The Journal*. While some of the changes made at the 1924 meeting were incorporated in the present edition of *Methods of Analysis*, it will be very desirable for the members of the Committee on Editing Methods of Analysis, at least, to have available at the time of the next revision, a concise statement of the changes made at the 1924 meeting similar in all respects to the compilations now in print of the changes made at the 1925 and 1926 meetings. Such a compilation, with notations in the margin of the manuscript, or elsewhere, to show which of the changes were incorporated in the present edition of *Methods of Analysis*, will be distributed in typewritten form to all the members of the committee. With this copy and the similar printed compilations of the changes made at the 1925, 1926, and following meetings, it will be a comparatively easy task to assemble at any time all the changes that have been made since July 1, 1924, the date of the revision of the present edition of *Methods of Analysis*.

For several years the association has had no referees on the subjects of wines, distilled liquors, and beers, and some criticism resulted from printing the chapters on these subjects in the present edition of *Methods of Analysis* in substantially the same form in which they appeared in the previous, or 1920, edition. This matter has recently been brought to the

¹ *This Journal*, 1926, 9: 27.

² *Ibid.*, 26.

³ *Ibid.*, 99.

attention of the Executive Committee with the recommendation that if any or all of these chapters are to be retained in the next edition, a referee or referees be appointed at this meeting for the purpose of having such changes made in these chapters as may appear to be desirable. The report of the action of the Executive Committee on the matter will be made by the secretary.

Last year, as may be recalled, the chairman of this committee and Julius Hortvet, the General Referee on Dairy Products, were appointed a committee with power to act, in cooperation with the American Public Health Association, in getting out a revised edition of Standard Methods of Milk Analysis. The 1923 edition of this pamphlet was prepared jointly by the two associations and thus included both bacteriological and chemical methods for the examination of milk. The material had been compiled for the new edition before Hortvet's death and there remained only the work of seeing it through the press. The 1927 edition is now in print, and copies are available for inspection in the registration room. The present edition, like that of 1923, has paper covers, although it was intended to have the 1927 edition in hard covers. Evidently there was a misunderstanding on the part of the printer, and it is probable that the American Public Health Association will have the edition rebound in hard covers.

R. W. BALCOM.

J. W. SALE.

B. B. ROSS.

G. W. HOOVER.

W. H. MACINTIRE.

Approved.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FORTY-THIRD ANNUAL CONVENTION, OCTOBER 31–NOVEMBER 2, 1927.¹

I. FERTILIZERS.

(1) The calcium chloride method for the preparation of ammonium citrate solution [p. 4, 13 (2)] was deleted (final action).

(2) In the official gravimetric method for the determination of total phosphoric acid, the words "Nearly neutralize with strong hydrochloric acid" (p. 3, 7, line 12) were deleted, and the words "Neutralize with strong hydrochloric acid, using litmus paper or bromthymol blue as indicator" were substituted therefor (first action).

(3) In the official gravimetric method for the determination of total phosphoric acid, the words, "burn first at a low heat and then ignite intensely until white or grayish white" (p. 3, 7, line 17), were deleted

¹ As compiled by the Committee on Editing Methods of Analysis, R. W. Balcom, Chairman. Unless otherwise stated, all references in this report are to *Methods of Analysis*, A. O. A. C., 1925.

and the directions, "burn first at a low heat and ignite to constant weight, preferably in an electric furnace, at 950°-1000°C.", were substituted therefor (first action).

(4) The words, "dilute to 1 liter", in the second of the alternative methods for the preparation of magnesia mixture [p. 2, 5 (c), last line], were deleted, and the words, "proceed as in (1)", were substituted therefor (final action).

(5) A third alternative method for the preparation of magnesia mixture [p. 2, 5 (c)] reading as follows: "(3) Dissolve 55 grams of crystallized magnesium chloride ($MgCl_2 \cdot 6H_2O$) in water, add 140 grams of ammonium chloride, and dilute to 870 cc. Add strong ammonium hydroxide to each required portion of the solution just before using, at the rate of 15 cc. per 100 cc. of solution", was adopted (first action).

(6) The absolute or cupric oxide method for the determination of total nitrogen (p. 9) was deleted from the methods for fertilizers (final action).

(7) The Jones method for the determination of nitric and ammoniacal nitrogen in mixed fertilizers containing cyanamide and/or urea was adopted as a tentative method. The method is as follows:

NITRIC AND AMMONIACAL NITROGEN.

Jones Method.—Tentative.

(Use when cyanamide or urea is present.)

PREPARATION OF SOLUTION.

Place 4 grams of the sample in a 150 cc. beaker, add 40 cc. of water, stir, allow to settle, and then decant the supernatant liquid through a filter into a 200 cc. volumetric flask. By means of a jet of water, transfer any residue remaining in the beaker to the filter and wash with successive portions of water, allowing each portion to pass through the filter before adding more, until the filtrate measures nearly 200 cc. Make up to the mark with water and mix.

DETERMINATION OF AMMONIACAL NITROGEN.

Place 25 cc. of the solution, prepared as directed under preparation of solution, in a 500-600 cc. Kjeldahl distillation flask with 150 cc. of water. Add 5 grams of heavy magnesium oxide, and proceed as directed in the official magnesium oxide method for the determination of ammoniacal nitrogen (p. 11).

DETERMINATION OF NITRIC NITROGEN.

(Aliquots for (a) and (b) must be taken from the same solution and run synchronously.)

(a) Place 25 cc. of the solution, prepared as directed under preparation of solution, in a 500-600 cc. Kjeldahl distillation flask, and add 25 cc. of water, 10-12 perforated glass beads (3-5 mm. in diameter), 2 grams of reduced iron, and 10 cc. of dilute sulfuric acid (1 + 1). Rotate the flask slowly and when the violence of any reaction that occurs has moderated, place on a hot plate and boil gently for 5 minutes. Remove the flask from the hot plate, add 40 cc. of water, cool, and add 100 cc. of 42° Baumé sodium hydroxide solution (555 grams of sodium hydroxide in 1 liter of water). Immediately connect the

flask by means of a Kjeldahl connecting bulb with a condenser, the tip of which extends below the surface of a measured quantity of standard acid in a receiver, and distil until 150–160 cc. of distillate has passed over and the last runnings are neutral to litmus paper. Titrate with standard alkali solution, using cochineal or methyl red indicator. The calculated nitrogen content of the distillate represents the nitrogen originally present in the form of nitrates and ammonium salts and that in any ammonia that may have been produced by this treatment from other nitrogenous compounds, such as protein, cyanamide, and urea.

(b) Proceed exactly as directed in (a) but do not add any reduced iron. The calculated nitrogen content of the distillate represents the nitrogen originally present in the form of ammonium salts and that in any ammonia that may have been produced by this treatment from other nitrogenous compounds, such as protein, cyanamide, and urea.

Subtract the quantity of nitrogen found in (b) from that found in (a) and from the result calculate the percentage of nitrogen (nitrate nitrogen) in the sample.

(8) The official zinc-iron method for the determination of nitric and ammoniacal nitrogen (p. 11, 35), which was placed under the sub-heading "Nitrogen in Nitrate Salts" (p. 12) at the 1925 meeting, was dropped (first action).

(9) The use of the official reduced iron method for the determination of nitric and ammoniacal nitrogen (p. 11, 34) was restricted by inserting the sentence, "Applicable only in the absence of cyanamide and urea" (first action).

(10) The official method for the determination of water-insoluble organic nitrogen soluble in neutral permanganate (p. 12) was amended by inserting the word "water-insoluble" before the word "nitrogen" in line 3 of paragraph 38 (first action).

(11) In the official method for the determination of water-insoluble organic nitrogen distilled from alkaline permanganate (p. 12) the directions in paragraph 40 for the preparation of the alkaline permanganate solution were changed to read as follows: "Dissolve 25 grams of potassium permanganate in hot water and, separately, 150 grams of sodium hydroxide in cold water; combine the solutions when cold and dilute to 1 liter. Discard any permanganate solutions that have become green in color" (first action).

(12) The directions for the preparation of sample for the determination of water-insoluble organic nitrogen distilled from alkaline permanganate (p. 13) were amended by adding to paragraph 41 (a) the following: "When it is found necessary to use 4 or more grams of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed" (first action).

(13) The directions for the determination of water-insoluble organic nitrogen distilled from alkaline permanganate (p. 13, 42) were changed (first action) to read as follows:

DETERMINATION.

Dry the residue remaining after treatment of the material as described in 41 at a temperature not exceeding 80°C. and transfer from the filter to a 500–600 cc. Kjeldahl distillation flask, loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers. Add 20 cc. of water, 15–20 small glass beads or fragments of pumice stone, a drop of mineral lubricating oil weighing not more than 50 mg., and 100 cc. of alkaline permanganate solution. Connect with an upright condenser to the lower end of which has been attached a 100 cc. graduated cylinder containing standard acid and so arranged as to receive the distillate below the surface of the acid or otherwise so trapped as to prevent loss of ammonia fumes. Digest slowly with a very low flame for 30 minutes, barely below distillation point, using coarse wire gauze and asbestos paper between the flask and flame. Gradually raise the temperature and, after all danger from frothing has passed, distil 95 cc. in 60 minutes (plus or minus 5 minutes), controlling the distillation so that approximately 24 cc. of distillate is obtained in each 15 minute period. Conduct the first part of the distillation over a bare flame but use wire gauze 10 minutes before completion to avoid breaking the flask. Transfer the distillate to an Erlenmeyer flask or to a beaker and titrate with standard alkali, using cochineal or methyl red indicator. When a tendency to froth is noticed, lengthen the digestion period, and no trouble will be experienced when the distillation is begun. During the digestion gently rotate the flask occasionally, particularly if the material shows a tendency to adhere to the sides.

The nitrogen thus obtained is the active water-insoluble organic nitrogen. If the active water-insoluble nitrogen is found to be less than 55 per cent of the total water-insoluble organic nitrogen present, it is recommended that a second portion of the sample be prepared as directed under 41 (a). Dry the residue below 80°C., transfer from the filter to a Kjeldahl flask as directed above, and determine the nitrogen as directed under 19 or 22. Recalculate the percentage of active water-insoluble nitrogen on the basis of the quantity of water-insoluble nitrogen thus found.

(14) The following was adopted as a tentative method for the determination of chlorine in fertilizers:

CHLORINE.—TENTATIVE.

REAGENTS.

(a) *Standard silver nitrate solution.*—Dissolve about 5 grams of pure recrystallized silver nitrate in water and dilute to 1 liter. Standardize against pure, dry sodium chloride and adjust so that 1 cc. of the solution is equivalent to 0.001 gram of chlorine.

(b) *Potassium chromate indicator.*—Dissolve 5 grams of potassium chromate in 100 cc. of water.

DETERMINATION.

Place 2.5 grams of the sample on a 11 cm. filter paper and wash with successive portions of boiling water until the washings amount to nearly 250 cc., collecting the filtrate in a 250 cc. volumetric flask. Cool, dilute to the mark with water, and mix well. Pipet 50 cc. into a 150 cc. beaker, add 1 cc. of the potassium chromate indicator, and titrate with the standard silver nitrate solution until the color produced by silver chromate appears as a permanent red.

II. SOILS.

No additions, deletions, or other changes.

III. AGRICULTURAL LIMING MATERIALS.

(1) A method for the determination of the caustic value of lime was adopted as an official method (first action). (This method will be printed in a later number of the *Journal*.)

(2) The tentative methods for the determination of calcium oxide in burnt and hydrated lime (p. 36, 5 and 6) were deleted.

IV. PLANTS.

No additions, deletions, or other changes.

V. INSECTICIDES AND FUNGICIDES.

(1) The official method for the determination of cyanogen in sodium and potassium cyanides (p. 65) was dropped (final action).

(2) The official method for the determination of chlorine in sodium and potassium cyanides (p. 65) was dropped (final action).

(3) The tentative method for the determination of cyanogen in sodium and potassium cyanides¹ was made official (final action).

(4) The tentative methods I and II for the determination of chlorine in sodium and potassium cyanides² were made official (final action).

(5) The tentative method for the determination of cyanogen in calcium cyanide³ was made official (final action).

(6) The tentative methods I and II for the determination of chlorine in calcium cyanide³ were made official (final action).

(7) The official method for the determination of moisture in soap (p. 65) was dropped (final action).

(8) The tentative xylene distillation method for the determination of water in soap⁴ was made official (final action).

(9) The tentative methods for the determination of (a) water, (b) total oil, and (c) ash in mineral oil-soap emulsions⁵ were made official (final action).

(10) The tentative method for the determination of soap in mineral oil-soap emulsions⁶, with the statement or note "In this method error will result if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid" appended, was made official (final action). First action toward making this method official was taken in 1926, and the same note was inserted at that time.

(11) The tentative method for the determination of unsulfonated residue in mineral oils⁷ was amended by striking out the third sentence, which reads "In lieu of measuring, determine the specific gravity of the

¹ *This Journal*, 1927, 10: 27.

² *Ibid.*, 28.

³ *Ibid.*, 29.

⁴ *Ibid.*, 1926, 9: 27.

⁵ *Ibid.*, 28, 29.

⁶ *Ibid.*, 28.

⁷ *Ibid.*, 1927, 10: 30.

oil and weigh the equivalent of 5 cc. into the bottle", and substituting therefor the following: "If greater accuracy is desired, the measured charge may be weighed and its exact volume calculated from the weight and specific gravity of the oil".

VI. TANNING MATERIALS.

No additions, deletions, or other changes.

VII. LEATHERS.

No additions, deletions, or other changes.

VIII. WATERS, BRINE, AND SALT.

No additions, deletions, or other changes.

IX. FEEDING STUFFS.

The following method for the detection of dried buttermilk in feeding stuffs was adopted as a tentative method.

DETECTION OF DRIED BUTTERMILK.—TENTATIVE.

Mount in water, upon a clean glass slide, about 5 mg. of that portion of the feed which passes through a 40-mesh sieve. Spread the material uniformly and keep for 5-10 minutes upon a level surface in a warm place to dry. When dry, immerse the slide in xylol or gasoline for 1 minute, drain, and dry again. Next immerse the slide in 90 per cent alcohol for at least 1 minute and then in a fresh aqueous solution of methylene blue, allowing it to remain in the latter solution from 5-60 seconds. Rinse the slide in water and decolorize in alcohol for several seconds, watching so that decolorization does not proceed too far. Dry, and examine the slide with the microscope, using a 1.9 mm. (1/12 in.) oil-immersion objective without a cover glass. If many blue-stained bacilli are found, dried buttermilk is present in the feed.

X. PRESERVATIVES AND ARTIFICIAL SWEETENERS.

No additions, deletions, or other changes.

XI. COLORING MATTERS IN FOODS.

No additions, deletions, or other changes.

XII. METALS IN FOODS.

No additions, deletions, or other changes.

XIII. SUGARS AND SUGAR PRODUCTS.

The first three lines (p. 195) containing the reference to Meissl's table and also the table (p. 448, 10) were deleted (first action).

XIV. FRUITS AND FRUIT PRODUCTS.

The title "Chlorides.—Official" (p. 211, 12) was changed to "Chlorine in Ash.—Official".

XV. CANNED VEGETABLES.

No additions, deletions, or other changes.

XVI. CEREAL FOODS.

FLOUR.

(1) The tentative method for sampling flour¹ was adopted as an official method (first action).

(2) The air-oven method for the determination of total solids and moisture (indirect method) in flour² with the word "routine" deleted from the title³ was made official (final action).

(3) The acid hydrolysis method for the determination of fat in flour⁴ was made official (final action).

(4) The F. A. C. method for the determination of unsaponifiable matter in fats and oils⁵, as modified and adopted at the 1926 meeting⁶, was adopted as a tentative method for the determination of the unsaponifiable matter in the fat of flour, but the determination is made upon the extract obtained from 5 grams of flour as directed in the method for the determination of lipoids, instead of upon 5 grams of fat.

(5) The tentative method for the determination of the hydrogen-ion concentration of flour⁷ was amended by adding to the last sentence, "using electrodes and a potentiometric set-up that have been checked through the use of a buffer solution of known hydrogen-ion concentration", and the amended method adopted as an official method (first action).

(6) The title "Gluten" (p. 227) was changed to "Crude Gluten".

(7) The sub-title "Quantitative Method.—Tentative" (p. 227, 16) was amended to read "Quantitative Method.—Tentative (results are approximate)".

(8) The following method for the determination of starch in flour was adopted as a tentative method.

STARCH.—TENTATIVE.

REAGENT.

Dilute hydrochloric acid.—Mix approximately equal volumes of strong hydrochloric acid and water and adjust so that 100 cc. of the solution contains 20.5–21.0 grams of hydrogen chloride.

DETERMINATION.

Weigh accurately a sufficient quantity of the finely ground sample to represent 0.5 to 1.0 gram of starch. Transfer to a funnel fitted with a 9 cm., S. and S. 589 white ribbon or Whatman No. 40 filter paper and extract four times with each of the follow-

¹ *This Journal*, 1926, 9: 39.

² *Ibid.*, 40.

³ *Ibid.*, 1927, 10: 33.

⁴ *Ibid.*, 1926, 9: 41.

⁵ *Ibid.*, 45.

⁶ *Ibid.*, 1927, 10: 35.

⁷ *Ibid.*, 33.

ing solvents in the order named: ether, 70 per cent (by volume) alcohol, and water. Transfer the drained filter and contents to a 50 cc. beaker. Add 1 cc. of the cold hydrochloric acid reagent; tamp the material with a stirring rod having a flattened end; and continue adding the acid gradually, with constant tamping and stirring, until the filter paper and sample are disintegrated to a smooth suspension. Transfer to a 100 cc. wide-mouth volumetric flask, rinsing with the hydrochloric acid reagent from a wash bottle. Fill to the mark with the same acid and then add 0.5 cc. more to compensate for the volume occupied by the filter paper. Mix and allow to stand for 3-5 minutes, shaking occasionally. During the treatment with acid the temperature should not exceed 22°C. Filter through a Gooch crucible prepared with a dry mat of ignited asbestos and filled two-thirds full with dry, fluffy, ignited asbestos. Pipet 50 cc. of the filtrate into a 200 cc. beaker (tall form) containing 115 cc. of 95 per cent (by volume) alcohol. (To prevent hydrolysis this last step must be completed within 35 minutes of the initial contact of the acid with the starch.) Allow the pipet to deliver completely and then stir with a whipping motion for 1 minute to flocculate the precipitated starch. Allow to stand for not longer than 5 minutes and then decant the supernatant liquid, which is somewhat turbid, through a weighed Gooch crucible that has been fitted with a thin pad of ignited asbestos. Wash the precipitate by decantation, using successively two 15 cc. portions each of 70 per cent (by volume) and 95 per cent (by volume) alcohol, breaking up the precipitate with a stirring rod during each washing. Decant each portion through the crucible and finally transfer the starch completely by means of a jet of 95 per cent (by volume) alcohol. Dry the crucible and contents to constant weight, which requires about 1½ hours at 130°C. or 5 hours in a vacuum oven at 100°C. Allow the crucible to remain uncovered during drying but replace the cover before removing the crucible from the oven, because the starch is extremely hygroscopic. Place in a desiccator charged with phosphorus pentoxide or with ignited calcium oxide and weigh immediately upon becoming cool.

(9) The factors for the conversion of the percentages of nitrogen into percentages of protein in wheat ($N \times 5.83$), wheat bran ($N \times 6.31$), wheat endosperm ($N \times 5.70$), and wheat embryo ($N \times 5.80$), as suggested by Jones¹, were adopted as the factors for such calculations.

(10) The method for the determination of moisture in flour (p. 225, 1) was deleted (final action).

ALIMENTARY PASTES.

(1) The tentative acid hydrolysis method for the determination of fat in flour², modified to read as follows, was adopted as an official method for the determination of fat in alimentary pastes (first action).

FAT (ACID HYDROLYSIS METHOD).—TENTATIVE.

Place 2 grams of the sample in a Röhrig or Mojonner fat extraction tube, add 2 cc. of 95 per cent (by volume) alcohol, and shake so as to moisten all particles. (The moistening of the sample with alcohol prevents lumping on addition of the acid.) Add 10 cc. of dilute hydrochloric acid (25 + 11), mix well, set the tube in a water bath held at 70°-80°C., and shake at frequent intervals for 30-40 minutes. Fill to the mark with 95 per cent (by volume) alcohol and cool. Add 25 cc. of ethyl ether and shake the mixture well. Then add 25 cc. of redistilled petroleum ether (b. p. below

¹ *Cereal Chem.*, 1926, 3: 194.

² *This Journal*, 1926, 9: 41.

60°C.) and mix well. Let stand until the upper liquid is practically clear and proceed from this point as directed in the acid hydrolysis method for the determination of fat in flour.

(2) The method for the determination of lipoids and lipid phosphoric acid (P_2O_5) in flour¹ was adopted as an official method for these determinations in alimentary pastes (first action).

(3) The F. A. C. method for the determination of unsaponifiable matter in fats and oils², as modified and adopted at the 1926 meeting³, was adopted as a tentative method for the determination of the unsaponifiable matter in the fat of alimentary pastes, but the determination is made upon the extract obtained from 5 grams of alimentary paste, as directed in the method for the determination of lipoids, instead of upon 5 grams of fat.

XVII. MEAT AND MEAT PRODUCTS.

In the official method for the determination of total nitrogen in meat (p. 237, 6) the last sentence, "In the Kjeldahl and Gunning methods digest with sulfuric acid for at least 4 hours; in the Kjeldahl-Gunning-Arnold method, for 2 hours after the mixture has become clear", was deleted (first action).

XVIII. GELATIN.

No additions, deletions, or other changes.

XIX. DAIRY PRODUCTS.

BUTTER.

(1) The words "or mix" in the second line of the official directions for the preparation of sample (p. 276, 67) were deleted (first action).

(2) The following method for the determination of moisture, fat, and salt in butter was adopted as a tentative method.

MOISTURE, FAT, AND SALT.—TENTATIVE.

APPARATUS.

Specially prepared Gooch crucible.—Prepare a Gooch crucible of about 30 cc. capacity with a 0.1 gram pad of asbestos and place thereon 20 grams of R. R. alundum, 90 mesh. (This is crystalline alumina especially prepared for carbon determinations. After use, the crucible is re-prepared for further use by igniting in a muffle, washing with water, and drying at 100°–105°C.).

DETERMINATION.

Weigh accurately in the weighed, specially prepared Gooch crucible 1.0–1.5 grams of the prepared sample, dry for 2 hours at 100°–105°C., cool, weigh, and calculate the percentage loss in weight as moisture. Extract the fat from the dried sample by placing the Gooch crucible in a closed-system extraction apparatus and extracting for

¹ *This Journal*, 1926, 9: 40.

² *Ibid.*, 45.

³ *Ibid.*, 1927, 10: 35.

30–40 minutes with carbon tetrachloride. Adjust the heat so that the solvent drops into the crucible at the same rate as the crucible drains and keep the crucible nearly full of the solvent. When the extraction is complete, remove most of the solvent remaining in the crucible by applying suction for a few seconds. Dry the crucible for 30 minutes at 100°–105°C., cool, weigh, and calculate the percentage of non-fat solids. Calculate the percentage of fat by subtracting the sum of the percentages of moisture and non-fat solids from 100.

If it is desired to determine the salt, wash it out of the non-fat solids with water and titrate the aqueous solution with standard silver nitrate solution, using potassium chromate indicator.

CHEESE.

The following methods for the detection and determination of tartaric and citric acids in cheese were adopted as tentative methods:

TARTARIC ACID.

Qualitative Test.—Tentative.

To 5 grams of the ground cheese, add 40 cc. of water at a temperature of about 50°C., and shake until the cheese is thoroughly broken up. Add 3 cc. of a 1 per cent sulfuric acid solution, shake vigorously, add 2 cc. of a 20 per cent solution of phosphotungstic acid, and shake again vigorously. Let stand for 5 minutes and filter. To 25 cc. of the filtrate add sufficient saturated barium hydroxide solution to make alkaline and 25 cc. of 95 per cent (by volume) alcohol. Shake vigorously and allow to settle. Filter through a Büchner funnel, using light suction, and wash the residue on the filter several times with water. Transfer a portion of the paste to a small evaporating dish and dry on the steam bath. Add a few cubic centimeters of concentrated sulfuric acid, a few crystals of resorcin, and heat slowly. If tartaric acid is present, a rose-red color that is slowly discharged on dilution with water is produced.

Quantitative Method.—Tentative.

REAGENTS.

(a) *Approximately 2 per cent solution of hydrochloric acid.*—Dilute 47 cc. of strong hydrochloric acid to 1 liter with water.

(b) *Sodium oxalate solution.*—Dissolve 2 grams of sodium oxalate in 100 cc. of water.

(c) *Potassium chloride wash solution.*—Dissolve 15 grams of potassium chloride in 100 cc. of water and add 20 cc. of 95 per cent (by volume) alcohol.

(d) *Tartaric acid solution.*—Dissolve 1.5 grams of pure tartaric acid in previously boiled and cooled water and dilute to 100 cc. at 20°C. Titrate with 0.1 *N* sodium hydroxide solution to determine the quantity of tartaric acid in 10 cc. of the solution.

(e) *Finely powdered potassium chloride.*

DETERMINATION.

Weigh 25 grams of the ground cheese into a 500 cc. wide-mouth salt bottle and add, 25 cc. at a time, 100 cc. of water at a temperature of 50°–60°C., shaking vigorously after each addition. If necessary, continue the shaking until the cheese is thoroughly broken up. Then add 25 cc. of the sodium oxalate solution and shake vigorously for 1 minute. Add 100 cc. of the hydrochloric acid solution, 25 cc. at a time, shaking vigorously after each addition. Add 50 grams of the powdered potassium chloride and shake for 5 minutes. To avoid churning, keep the mixture warm (at about 50°C.) during the shaking. Transfer the contents of the bottle, with the aid of water, to a 300 cc. volumetric flask, cool to 20°C., and make up to the mark with water. Mix

thoroughly; let stand for 10 minutes, with occasional shaking; and then filter through a dry folded filter, discarding the first few cubic centimeters of the filtrate. Disregard any opalescence and transfer 200 cc. of the filtrate to a 250 cc. volumetric flask. Neutralize with 1 *N* sodium hydroxide solution, using phenolphthalein indicator, and then add 5.2 cc. in excess. Make up to the mark with water, mix thoroughly, let stand for a few minutes, and filter through a dry folded filter, discarding the first few cubic centimeters of the filtrate. To 100 cc. of the filtrate in a 250 cc. beaker add, with constant stirring, 10 cc. of the tartaric acid solution, 2 cc. of glacial acetic acid, and 23 cc. of 95 per cent (by volume) alcohol. Cool in an ice bath, stir vigorously until the cream of tartar begins to crystallize, and let stand in a refrigerator overnight. Prepare a Gooch crucible, having a removable disk, with a pad of asbestos about 10 mm. thick. Decant most of the liquid through this filter, wash the precipitate into the crucible with the potassium chloride wash solution, and wash the beaker and precipitate three times, using 20–30 cc. of the wash solution in all. Place the asbestos and precipitate in the beaker in which the precipitation was made and wash the crucible with about 50 cc. of hot water. Heat the solution to boiling and titrate the hot solution with 0.1 *N* sodium hydroxide solution, using phenolphthalein indicator. Calculate the percentage of tartaric acid in the cheese by means of the formula

$$X = 14.26^1 [0.015 (B + 1.5) - A], \text{ in which}$$

A = grams of tartaric acid in 10 cc. of the tartaric acid solution reagent; and

B = cubic centimeters of 0.1 *N* sodium hydroxide solution required for the titration.

CITRIC ACID².

Qualitative Test.—Tentative.

To 10 grams of the ground cheese, add 20 cc. of water at a temperature of about 50°C. and shake vigorously until the cheese is thoroughly broken up. Add 20 cc. of dilute sulfuric acid (1 + 1), 2 cc. of a 20 per cent solution of phosphotungstic acid, and shake vigorously. Let stand for 5 minutes and filter. To 20 cc. of the filtrate add 10 cc. of bromine water and 5 cc. of potassium bromide solution and proceed with the oxidation as directed in the quantitative determination. Add sufficient ferrous sulfate solution to dissolve the precipitated manganese dioxide. If citric acid is present, a heavy white precipitate that settles rapidly is formed.

Quantitative Method.—Tentative.

REAGENTS.

(a) *Approximately 1 per cent solution of sulfuric acid.*—Dilute 6 cc. of concentrated sulfuric acid to 1 liter with water.

(b) *Sodium oxalate solution.*—Dissolve 2 grams of sodium oxalate in 100 cc. of water.

(c) *Finely powdered anhydrous sodium sulfate.*

(d) *Phosphotungstic acid solution.*—Dissolve 20 grams of phosphotungstic acid in water and dilute to 100 cc.

(e) *Potassium bromide solution.*—Dissolve 15 grams of potassium bromide in 40 cc. of water.

(f) *Potassium permanganate solution.*—Dissolve 5 grams of potassium permanganate in water and dilute to 100 cc.

(g) *Ferrous sulfate solution.*—Dissolve 20 grams of ferrous sulfate in 100 cc. of water containing 1 cc. of concentrated sulfuric acid.

(h) *Bromine water.*—A freshly prepared saturated solution.

¹ In this factor the concentration caused by the insoluble solids of cheese of average composition is also taken into consideration.

² Hartmann and Hillig. *This Journal*, 1927, 10: 264.

DETERMINATION.

Weigh 25 grams of the ground cheese into a 500 cc. wide-mouth salt bottle, and add, 25 cc. at a time, 100 cc. of water at a temperature of 50°–60°C., shaking vigorously after each addition. If necessary, continue the shaking until the cheese is thoroughly broken up. Then add 25 cc. of the sodium oxalate solution and shake vigorously for 1 minute. Add 100 cc. of the 1 per cent sulfuric acid solution, 25 cc. at a time, shaking vigorously after each addition. Add 3 cc. of the phosphotungstic acid solution, shake, add 25 grams of the powdered sodium sulfate, and shake for 5 minutes. To avoid churning, keep the mixture warm (at about 50°C.) during the shaking. Transfer the contents of the bottle with the aid of water to a 300 cc. volumetric flask, cool to 20°C., and make up to the mark with water. Mix thoroughly, let stand for 10 minutes with occasional shaking, and then filter through a dry folded filter, discarding the first few cubic centimeters of the filtrate. Heat 200 cc. of the filtrate to boiling and, while still hot, add 20 cc. of dilute sulfuric acid (1 + 1) and 2 cc. of the phosphotungstic acid solution. Mix and allow to stand for 15 minutes. With the aid of water transfer to a 250 cc. volumetric flask, cool to 20°C., make up to the mark with water, and filter through a dry folded filter. Transfer 100 cc. of the clear filtrate to a 500 cc. Erlenmeyer flask containing about 0.3 gram of washed and dried asbestos. Add 10 cc. of the bromine water and 5 cc. of the potassium bromide solution, mix thoroughly, and heat to 48°–50° C. Hold at this temperature for 5 minutes and then add 2.5 cc. of the permanganate solution. Shake and allow to stand for about 5 minutes. Cool the flask and contents to about 8°C., add 40 cc. of the cold ferrous sulfate solution, shake continuously for 5 minutes, and let the mixture stand overnight in the refrigerator. Decant the supernatant liquid through a Gooch crucible, measure the volume of the filtrate (a) and wash the precipitate into the crucible with this filtrate. Wash the precipitate with three successive 20 cc. portions of ice-cold dilute sulfuric acid (1 + 100), sucking dry after each addition, and finally with three successive 20 cc. portions of ice-cold water. Dry the precipitate to constant weight over sulfuric acid in a vacuum desiccator, protecting the precipitate from strong light or, to save time, dry in a current of air passed through sulfuric acid. Weigh, and remove the pentabromacetone by extracting first with three successive 20 cc. portions of 95 per cent (by volume) alcohol and then with three successive 20 cc. portions of ether. Dry and weigh the crucible. To the weight of the pentabromacetone add 0.004 gram for each 100 cc. of filtrate (a) to compensate for solubility of the pentabromacetone and multiply the result by 6.06 to obtain the percentage of anhydrous citric acid in the cheese. (In this factor consideration is taken of the concentration caused by the insoluble solids in 25 grams of cheese. It is assumed that the solids of cheese are almost insoluble under the conditions maintained and that the average process cheese contains about 60 per cent of solids. No allowance is made for variation in the salt or moisture contents or for variation in the specific volume of the solids, as such variations do not appreciably affect the results.)

MALTED MILK.

(1) The tentative method for the determination of moisture in malted milk (p. 275, 62) was deleted.

(2) The following, a slight modification of the method for the determination of moisture in cheese¹, was adopted as a tentative method for the determination of moisture in malted milk:

¹ *This Journal*, 1926, 9: 44.

MOISTURE.—TENTATIVE.**APPARATUS.**

As used in the official method for the determination of moisture in cheese.

DETERMINATION.

Weigh 1–1.5 grams of the sample into the previously weighed metal dish, cover tightly, and again weigh. Dry in the loosely covered dish, placed in direct contact with the metal shelf of the vacuum oven, to constant weight (approximately 5 hours) under a pressure not to exceed 100 mm. (4 inches) of mercury, at the temperature of boiling water. During the drying admit into the oven a slow current of air (about 2 bubbles per second), dried by passing through concentrated sulfuric acid. Discontinue the action of the vacuum pump and carefully admit dried air into the oven. Press the cover tightly into the dish, remove the dish from the oven, cool, and weigh. Calculate the percentage loss in weight as moisture.

(3) The following, a slight modification of the tentative method for the determination of fat in dried milk¹, was adopted as a tentative method for the determination of fat in malted milk.

FAT.—TENTATIVE.

Weigh accurately about 1 gram of the well-mixed sample into a small, lipped beaker. Add 1 cc. of water and mix well with a glass rod to form a thick liquid free from lumps. Add 10 cc. more of water, warm on the steam bath, and transfer to a Röhrig tube or similar apparatus. Cool, add 10 cc. of 95 per cent (by volume) alcohol, and mix. Add 25 cc. of ethyl ether and proceed with the extraction as in the official Roese-Gottlieb method for milk. Dissolve the dried fat in petroleum ether and determine the quantity of any insoluble residue that may be present. In case of whole milk and cream powders make a third extraction, using 15 cc. of each ether.

(4) The methods for malted milk were combined with the methods for dried milk under the sub-title "Dried Milks and Malted Milks" in the chapter on "Dairy Products" in *Methods of Analysis*.

DRIED MILK.

(1) The same modification of the method for the determination of moisture in cheese, adopted as a tentative method for the determination of moisture in malted milk (see above) was adopted as a tentative method for the determination of moisture in dried milk.

(2) The methods for dried milk were combined with the methods for malted milk under the sub-title "Dried Milks and Malted Milks" in the chapter on "Dairy Products" in *Methods of Analysis*.

ICE CREAM.

The Kjeldahl-Gunning-Arnold method for the determination of total nitrogen (p. 8, 24), with the first sentence changed to read "Place 4–5 grams of the sample to be analyzed in a digestion flask", was adopted as a tentative method for the determination of nitrogen in ice cream.

¹ *This Journal*, 1925, 8: 482.

XX. FATS AND OILS.

(1) The modified André-Cook method for the determination of acetyl value¹ was made official (final action).

(2) The method for the determination of acetyl value (p. 293) was deleted (final action).

XXI. BAKING POWDERS AND BAKING CHEMICALS.

(1) The tentative gasometric method for the determination of total carbon dioxide (p. 305, 8, 9, and 10), as amended¹, was made official (final action).

(2) The tentative gasometric method for the determination of residual carbon dioxide (p. 306, 12), as amended², was further amended by adding the sentence, "To prevent foaming, 1-3 drops of caprylic alcohol may be added to the baking powder in the decomposition flask", and the method was then made official (final action).

XXII. SPICES AND OTHER CONDIMENTS.

No additions, deletions, or other changes.

XXIII. VINEGARS.

No additions, deletions, or other changes.

XXIV. COFFEES.

No additions, deletions, or other changes.

XXV. TEAS.

No additions, deletions, or other changes.

XXVI. CACAO PRODUCTS.

(1) To correct an oversight, the word "Tentative" was inserted after "Cacao Shell" (p. 347, 29). The method for the determination of cacao shell given under this heading was adopted as a tentative method at the 1921 meeting³ but through inadvertence was not so designated in the 1925 edition of *Methods of Analysis*.

(2) To correct an oversight the word "Tentative" was inserted after "Coloring Matters" (p. 347, 28).

(3) The method for the determination of fat (p. 345, 14) was deleted (final action).

(4) The following method for the detection of coconut and palm kernel oils in cacao butter and in fat extracted from milk chocolate was adopted as a tentative method.

¹ *This Journal*, 1927, 10: 35.

² *Ibid.*, 36.

³ *Ibid.*, 1922, 6: 150.

DETECTION OF COCONUT AND PALM KERNEL OILS IN CACAO BUTTER AND FAT EXTRACTED FROM MILK CHOCOLATE.—TENTATIVE.**REAGENTS.**

(a) *Alcoholic potassium hydroxide solution.*—Dissolve 25 grams of potassium hydroxide in 200 cc. of alcohol.

(b) *Saturated salt solution.*—Prepare from common salt.

EXAMINATION OF CACAO BUTTER.

Saponify 5 grams of the sample with 15 cc. of the alcoholic potassium hydroxide solution and evaporate the alcohol on a steam bath. Run a blank on pure cacao butter at the same time. Add 5 cc. of water and again evaporate to remove the last trace of alcohol. Dissolve the soap in 100 cc. of water, cool to room temperature, and add, while stirring, 100 cc. of the saturated salt solution. Allow to stand for 15 minutes, stirring several times during this period, and then separate the soap by filtration, using a Büchner funnel. To 100 cc. of the filtrate add, while stirring, 100 cc. of the saturated salt solution and allow to stand for 15 minutes. Only a slight precipitate should appear. Filter, add to the filtrate a drop of phenolphthalein indicator, neutralize with dilute hydrochloric acid (1 + 3), and then add 0.5 cc. of this reagent in excess. If the sample consists of pure cacao butter, the solution, when acidified, will remain clear. If coconut or palm oil is present, the solution will become turbid or milky.

EXAMINATION OF FAT EXTRACTED FROM MILK CHOCOLATE.

Milk fat, if present in cacao butter subjected to this test, produces a turbidity, but a less intense turbidity than that produced by the same percentage of coconut or palm kernel oil. For example, cacao butter that contains 20 per cent of milk fat will show only an opalescence. For this reason when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply the percentage of lactose in the cacao product by 0.8 or the percentage of casein by 1.1 to obtain the percentage of milk fat in the product, and from this result calculate the percentage of milk fat in the total fat. Make up a mixture of cacao butter and milk fat in the proportions indicated by the calculations.

Test the fat extracted from the sample under examination, as directed for the examination of cacao butter, but use the prepared mixture of cacao butter and butter fat, instead of pure cacao butter, for the blank. If the fat being tested contains coconut oil or palm kernel oil, the last filtrate, when acidified, will be more turbid or milky than the blank.

XXVII. FLAVORING EXTRACTS.

(1) The following steam distillation method for the determination of oils of lemon, orange, and limes in corn and cottonseed oils and in mineral oil was adopted as an official method (first action).

OILS OF LEMON, ORANGE, AND LIMES, IN VEGETABLE AND MINERAL OILS.*By Steam Distillation.—Tentative.***APPARATUS.**

(a) *Steam generator filled with water.*—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask.*—Consists of a Kjeldahl flask of about 750 cc. capacity, with shortened neck, about 10 inches in height over all.

(c) *Spray tube.*—A glass tube with a small perforated bulb at the end. Passes through a rubber stopper and reaches to the bottom of the distillation flask.

(d) *Bent glass tube*.—About 8 mm. in diameter. Connects distillation flask to upright condenser. The shape of this tube allows the vapor condensing in the tube to return to the distillation flask.

(e) *Liebig condenser*.—With 20-inch water jacket.

(f) *Wilson receiving flask*.—Shaped like a Babcock test bottle but of much larger capacity and with a vertical glass outlet tube sealed on near the bottom. The upper end of the outlet tube is turned down. The capacity of the flask is about 250 cc. The neck may consist of a portion of a buret graduated from 0–25 cc. with top flared out. The outlet tube is about 3 mm. in diameter, and the end is at such a height that when the flask is filled with water the meniscus in the neck will be between the 0 and 1 cc. marks.

DETERMINATION.

Measure 100 cc. of the sample in a graduated cylinder and transfer to the distillation flask. Immerse the flask in a water bath and connect with the condenser by means of the bent glass tube. Fill the receiving flask with water and place under the condenser in such a way that the end of the condenser will be about 0.5 inch above the level of the water in the receiving flask. Place a 200 cc. graduated cylinder under the end of the outlet tube to catch the displaced liquid. Heat the water bath to boiling and pass steam through the sample until 200 cc. of liquid has been collected in the graduated cylinder.

Disconnect the apparatus, allow the receiving flask to stand for 15 minutes, or until separation of oil is complete, and read the volume of oil obtained. Calculate the percentage (by volume) of essential oil in the sample by dividing the reading by 0.90 for lemon oil in corn and cottonseed oils, 0.95 for orange oil in corn and cottonseed oils, and by 0.78 for distilled or expressed oil of limes in corn and cottonseed oil's. Where the menstruum is mineral oil, subtract 0.3 cc. from the reading before dividing by the factors 0.90, 0.95, and 0.78 for lemon oil, orange oil, and oil of limes, respectively.

(2) The following methods for the determination of anthranilic acid ester were adopted as official methods (first action).

ANTHRANILIC ACID ESTER.

Colorimetric Method.—Tentative.

(Use when sample contains not more than about 500 mg. per liter of anthranilic acid ester.)

REAGENTS.

(a) *Hydrochloric acid solution*.—Dilute 83 cc. of strong hydrochloric acid to 1 liter with water.

(b) *Sodium nitrite solution*.—Dissolve 2 grams of sodium nitrite in 100 cc. of water.

(c) *Saturated hydrazine sulfate solution*.—Contains about 3 grams of hydrazine sulfate in 100 cc. of water.

(d) *Sodium- α -naphthol-2-sulfonate solution*.—Dissolve 5 grams of the sulfonate in 100 cc. of water.

(e) *Sodium carbonate solution*.—Dissolve 25 grams of sodium carbonate in 75 cc. of water.

(f) *Standard solution of methyl anthranilate*.—Dissolve 0.25 gram of methyl anthranilate in 60 cc. of 95 per cent (by volume) alcohol and dilute with water to 250 cc.

APPARATUS.

(a) *Steam generator filled with water*.—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask*.—Consists of a Kjeldahl flask of about 750 cc. capacity, with shortened neck, about 10 inches in height over all.

(c) *Spray tube*.—A glass tube with a small perforated bulb at the end. Passes through a rubber stopper and reaches to the bottom of the distillation flask.

(d) *Connecting bulb*.—A Kjeldahl bulb with bent connecting tube.

(e) *Worm condenser*.—10 to 12 inches in length and with a glass tube that will reach to the bottom of the receiving flask sealed to the outlet.

(f) *Receiving flask*.—A 500 cc. Erlenmeyer flask.

DETERMINATION.

Place enough water in the receiving flask to just cover or seal the end of the extended condenser tube. Place 10–100 cc. of the sample of flavor in the distillation flask and add, if necessary, sufficient water to make the volume 100 cc. Insert stopper carrying the spray tube and connecting bulb and connect with the condenser and receiving flask. Immerse the distillation flask in a water bath to the level of the contents, and when the sample has attained the temperature of the nearly boiling bath connect with the steam generator and pass a rapid current of steam through the sample until about 300 cc. of distillate has been collected.

Disconnect the apparatus and wash out the condenser with a little water. Add to the distillate 25 cc. of the standard hydrochloric solution and 2 cc. of the sodium nitrite solution. Mix well and let stand for exactly 2 minutes. Add 6 cc. of the saturated solution of hydrazine sulfate and mix well for a minute, so that the liquid comes in contact with all parts of the flask that may have been touched by the solution when it contained free nitrous acid. Keep the liquid in the flask in rapid motion, add quickly 5 cc. of the sodium- α -naphthol-2-sulfonate solution, and then add immediately 15 cc. of the sodium carbonate solution. Dilute the colored solution to 500 cc. with water, mix, and compare the color of an aliquot with the color of a standard or set of standards, prepared as nearly as possible at the same time. Calculate and express results as milligrams of anthranilic acid ester, as methyl anthranilate, per liter of sample.

Gravimetric Method.—Tentative.

(Use when the sample contains more than about 500 mg. per liter of anthranilic acid ester.)

REAGENTS.

(a) *Hydrochloric acid solution*.—Dilute 83 cc. of strong hydrochloric acid to 1 liter with water.

(b) *Sodium nitrite solution*.—Dissolve 2 grams of sodium nitrite in 100 cc. of water.

(c) *α -naphthol solution*.—Dissolve 0.2 gram of α -naphthol in 100 cc. of 30 per cent (by volume) alcohol.

(d) *Sodium bicarbonate solution*.—Dissolve 8.4 grams of sodium bicarbonate in 100 cc. of water.

APPARATUS.

The same as in the colorimetric method.

DETERMINATION.

Place such a quantity of the sample of flavor as contains from 50–125 mg. of anthranilic acid ester in the distillation flask and dilute, if necessary, to 100 cc. with water. Subject the sample to steam distillation as directed in the colorimetric method, collecting about 400 cc. of distillate. (The water in the water bath should be near the

boiling point when the bath is placed under the distillation flask, and the steam generator should be boiling also, so that connection can be made immediately.)

Dilute the distillate to 500 cc., mix, and to a 200 cc. aliquot add 5 cc. of the hydrochloric acid solution and 5 cc. of the sodium nitrite solution. Mix well and let stand for 1 minute. Mix 25 cc. of the alpha-naphthol solution and 6 cc. of the sodium bicarbonate solution, pour the diazotized solution into the mixture, and let stand for 10 minutes. Fold two Whatman No. 1 or S. & S. No. 595 filter papers, 12.5 cm. in diameter, and determine the difference in their weights by placing one on each pan of the balance and counterpoising with added weights. Place the heavier inside the lighter paper, fit into a funnel, and moisten. Filter the mixture through this filter and wash the precipitate seven or eight times, using a total of about 100 cc. of water for this purpose. Fill the filter only to within 1 cm. of the top. Place the funnel carrying the filter and washed precipitate in an oven and dry for about 10 minutes at a temperature of 100°C. Then separate the filter papers and dry them separately for about 1 hour at the same temperature. Ascertain the difference in weights, dry again, re-weigh, and repeat this procedure until the difference in weights remains constant. From this constant difference in weights subtract the original difference in weights of the two filter papers and multiply the result by 0.4935 to obtain the weight of anthranilic acid ester, as methyl anthranilate. Calculate and express as grams per liter of sample.

XXVIII. WINES.

No additions, deletions, or other changes.

XXIX. DISTILLED LIQUORS.

No additions, deletions, or other changes.

XXX. BEERS.

No additions, deletions, or other changes.

XXXI. DRUGS.

ARSENICALS.

(1) The tentative method for the determination of arsenic in sodium cacodylate¹, slightly amended, was adopted as an official method (first action). As amended, the method is as follows:

ARSENIC IN SODIUM CACODYLATE.—TENTATIVE.

Transfer 0.2 gram of the sample, accurately weighed, to a Kjeldahl flask. Conduct a blank using the same quantities of reagents. Add 10 grams of potassium sulfate, 0.3 gram of starch, and 20 cc. of concentrated sulfuric acid. Digest over a low flame until frothing has ceased. Continue the digestion 4 hours or until the mixture is colorless. Cool, dilute with water, and transfer to a 500 cc. Erlenmeyer flask. Add sodium hydroxide solution (1 + 1) slowly until alkaline to litmus paper and acidify with sulfuric acid. Place the flask in water until thoroughly cooled, add 5 grams of sodium bicarbonate, and titrate with 0.1 *N* iodine solution.

One cc. of 0.1 *N* iodine solution is equivalent to 0.00375 gram of arsenic (As) or to 0.008 gram of anhydrous sodium cacodylate.

¹ *This Journal*, 1926, 9: 51.

(2) The following method for the determination of arsenic in iron methylarsenates was adopted as a tentative method.

ARSENIC IN IRON METHYLARSENATES.—TENTATIVE.

Transfer a suitable quantity of the sample (0.2 gram, if practicable) to a Kjeldahl flask. Conduct a blank using the same quantities of reagents. Add 10 grams of potassium sulfate, 0.3 gram of starch, and 20 cc. of concentrated sulfuric acid. Digest over a low flame until frothing has ceased and continue the digestion over a slightly higher flame until the mixture is colorless. Cool, and add 20 cc. of water. Dry the neck of the flask over a small flame; cool the contents; and add 30 grams of sodium chloride, 5 grams of ferrous sulfate, 1 gram of sodium bromide, and 25 cc. of strong hydrochloric acid. Then distil as directed in the method for the determination of arsenic in iron-arsenic tablets¹.

COCAINE.

The following methods for the determination of cocaine were adopted as tentative methods:

Method I.

Weigh accurately a sufficient quantity of the uniformly mixed sample to represent approximately 0.2 gram of the alkaloid. Dissolve in 20 cc. of cold water, add 2 drops of dilute hydrochloric acid, and transfer to a separatory funnel. Make alkaline to litmus with a freshly prepared saturated solution of sodium bicarbonate and shake out to exhaustion with petroleum ether (four 20 cc. portions are usually sufficient). Run the combined extract through a plug of absorbent cotton into a separatory funnel and wash the cotton with petroleum ether. Add a decided excess of 0.02 *N* sulfuric acid, accurately measured, and shake vigorously for several minutes. Separate the two layers and wash the petroleum ether with two 10 cc. portions of water, adding the washings to the acid solution. Titrate the excess of acid with 0.02 *N* alkali, using methyl red indicator, and reserve the titrated solution for the check determination described below. Each cubic centimeter of 0.02 *N* sulfuric acid required for combination with the alkaloid is equivalent to 0.006793 gram of cocaine hydrochloride.

As a check, add 10 cc. of 2.5 *N* sodium hydroxide solution to the titrated alkaloid solution and evaporate on the steam bath to a volume of about 10 cc. Cool, transfer the solution to a separatory funnel, and acidify with dilute hydrochloric acid. Extract the acid solution completely with successive portions of chloroform. Run the combined extracts through a plug of absorbent cotton and wash the cotton well with chloroform. Allow the chloroform solution to evaporate spontaneously in a weighed beaker, dry the residue in a vacuum desiccator for 2 hours, and weigh. From the weight of benzoic acid found calculate its equivalent of cocaine hydrochloride. One gram of benzoic acid is equivalent to 2.782 grams of cocaine hydrochloride. (The quantity of benzoic acid may be determined by titration, if desired.)

Method II.

Weigh accurately a sufficient quantity of the uniformly mixed sample to represent approximately 0.2 gram of the alkaloid. Transfer to a small separatory funnel and dissolve with the minimum quantity of water required for solution. Make the solution slightly alkaline with ammonium hydroxide and extract with successive small portions of peroxide-free ether until the alkaloid is completely removed from the aqueous solution, using Mayer's reagent for the test. Combine the ether extracts, remove the greater part of the ether by evaporation on the steam bath, and allow the remainder

¹ *This Journal*, 1927, 10: 45.

of the ether to evaporate spontaneously at room temperature. Dissolve the residue in a few cubic centimeters of neutral alcohol, add 20 cc. of 0.05 *N* sulfuric acid, and titrate the excess of acid with 0.02 *N* sodium hydroxide, using methyl red indicator. Each cubic centimeter of 0.05 *N* sulfuric acid required for combination with the alkaloid is equivalent to 0.01698 gram of cocaine hydrochloride, $C_{17}H_{21}O_4N.HCl$.

IPECAC PREPARATIONS.

The following methods for the determination of ipecac alkaloids in fluid extract of ipecac were adopted as tentative methods:

Automatic Extraction Method.—Tentative.

PREPARATION OF SOLUTION.

Pipet 20 cc. of the fluid extract into a 100 cc. volumetric flask, add approximately 5 cc. of normal sulfuric acid, and with the aid of an air blast evaporate on a steam bath to a volume of about 10 cc. Then, while rotating the flask, add about 30 cc. of water, cool to room temperature, and make up to the mark with water. Allow to stand for 5 minutes and filter through a dry filter, rejecting the first few cubic centimeters of the filtrate.

DETERMINATION.

Pipet 20 cc. of the filtrate, prepared as directed above, into the automatic extractor¹, which has been fitted to a 200 cc. Erlenmeyer flask. Add 2 cc. of 8 per cent ammonium hydroxide solution and about 25 cc. of peroxide-free ether. Shake gently to prevent the deposition of any solid matter on the bottom of the extractor and then add peroxide-free ether until about 50 cc. overflows into the flask. Heat the flask on a steam bath (not electric hot plate) and extract for 2 hours or until the extraction is complete. Separate the ether from the aqueous layer and add it to the main concentrate in the flask. Evaporate the combined ether extract on a steam bath, add 2–3 cc. of absolute alcohol, and repeat the evaporation to remove all traces of ammonia. Warm the alkaloidal residue with 2–3 cc. of neutral alcohol on the steam bath to insure complete solution; add 10 cc. of 0.1 *N* sulfuric acid, accurately measured; dilute with about 20 cc. of water; and titrate the excess of acid with 0.02 *N* alkali, using methyl red indicator. Each cubic centimeter of 0.1 *N* sulfuric acid required for combination with the alkaloids is equivalent to 0.024 gram of ether-soluble alkaloids of ipecac.

Hand Extraction Method.—Tentative.

(This method is sometimes more rapid than the automatic extraction method and yields results almost as high.)

Pipet 20 cc. of the filtrate, prepared as directed under preparation of solution in the automatic extraction method, into a separatory funnel. Add 2 cc. of 8 per cent ammonium hydroxide solution and extract the solution with equal volumes of peroxide-free ether until extraction is completed (at least eight times), using Mayer's reagent as a test. Wash the combined ether extracts in a second separatory funnel with about 10 cc. of water, and then wash this wash water with a little peroxide-free ether, adding the ether washings to the main solution. Transfer the ether solution to an Erlenmeyer flask (a 200 cc. flask is a convenient size), and evaporate the ether in a steam bath with the aid of a blast of air. Add 2–3 cc. of absolute alcohol and repeat the evaporation to remove all traces of ammonia. Warm the alkaloidal residue with 2–3 cc. of neutral alcohol to insure complete solution, and titrate as directed in the automatic extraction method.

¹ Palkin, Murray, and Watkins. *Ind. Eng. Chem.*, 1925, 17: 612.

MERCURIALS.

The following method for the determination of calomel in tablets was adopted as a tentative method:

CALOMEL IN TABLETS.—TENTATIVE.

REAGENTS.

- (a) *Purified iodine*.—Prepare as directed on p. 379, 2 (a).
- (b) *Standard sodium thiosulfate solution*.—Prepare as directed on p. 379, 2 (b).
- (c) *Standard iodine solution*.—Dissolve about 14 grams of iodine in a solution containing 18 grams of potassium iodide in 100 cc. of water and dilute to 1 liter. Standardize this solution against the standard sodium thiosulfate solution.

DETERMINATION.

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of the well-mixed, pulverized sample to represent 3–4 grains (0.19–0.26 gram) of calomel. Transfer to a 200 cc. glass-stoppered Erlenmeyer flask, add about 50 cc. of water, acidify with acetic acid, and, after solution of the soluble fillers, filter through a small filter paper. Wash well with water and return the paper and the insoluble material upon it to the flask. Add 2.5 grams of potassium iodide, 10 cc. of water, and then 30 cc. of the standard iodine solution. Allow the mixture to stand, with frequent and fairly vigorous agitation, for about 1½ hours, or until solution of the calomel is complete. Titrate with the standard thiosulfate solution and add about 1 cc. in excess. Then titrate back with the standard iodine solution, using starch indicator [p. 48, 3 (e)], until a permanent blue color is obtained.

One cc. of 0.1 *N* iodine solution is equivalent to 0.02361 gram of calomel.

PYRAMIDON.

(1) The tentative extraction method (Method I)¹ for the determination of pyramidon was deleted.

(2) The tentative hydrochloride method (Method II)² for the determination of pyramidon was deleted.

(3) The following method for the determination of pyramidon was adopted as a tentative method.

Quantitative Method.—Tentative.

PREPARATION OF SAMPLE.

Pulverize the material in a mortar and mix the powder thoroughly.

DETERMINATION.

Place 1 gram of the sample in a 100 cc. volumetric flask, add 60 cc. of normal hydrochloric acid, and shake for several minutes to insure complete solution of the pyramidon. Make up to the mark with normal hydrochloric acid. Filter, if not clear, through a dry filter, rejecting the first part of the filtrate. Pipet a 20 cc. aliquot portion of the solution, or filtrate, into a separatory funnel; make distinctly alkaline with either ammonium hydroxide or with dilute sodium hydroxide; and shake out with 20, 15, 10, 10, and 5 cc. portions of chloroform. Combine the chloroform extracts in a second separatory funnel and wash with 2 cc. of water. Filter the chloroform solution into a weighed beaker through a pledget of cotton saturated with chloroform. Extract the wash water

¹ *This Journal*, 1925, 8: 546.

² *Ibid.*, 547.

with 5 cc. of chloroform and add this to the combined chloroform extracts. Evaporate the united chloroform extracts just to dryness on a water bath with the aid of an electric fan and dry the residue in an oven at the temperature of boiling water for 10 minutes. Cool in a desiccator, and weigh as pyramidon. Identify the pyramidon by means of its melting point or by qualitative tests, or both.

Microchemical Tests for Alkaloids.

The following microchemical tests were adopted as tentative methods for the identification of cocaine, codeine, morphine, and strychnine:

REAGENTS.

(a) *Marme's reagent*.—Dissolve 3 grams of cadmium iodide in 18 cc. of water containing in solution 6 grams of potassium iodide.

(b) *Wagner's reagent*.—Dissolve 1 gram of iodide in 100 cc. of water containing in solution 5 grams of potassium iodide.

(c) *Platinic chloride solution*.—Dissolve 5 grams of platinic chloride ($H_2Pt Cl_6$) in 100 cc. of water.

PREPARATION OF SAMPLES.

(a) *Controls*.—Dissolve 1 mg. of the pure alkaloidal salt in 2 drops of water to make an approximately 1-100 solution.

(b) *Alkaloids in compounds*.—Separate the alkaloid in pure form by extracting it from ammoniacal solution with a suitable immiscible solvent, and evaporate the solvent. To 1 mg. of the residue add, drop by drop, 0.1 *N* hydrochloric acid, avoiding an excess of acid, and dilute with water, if necessary, to approximately the same alkaloidal concentration as in (a).

(d) *Hypodermic tablets*.—Dissolve a portion of a tablet in water and dilute with water to approximately the same alkaloidal concentration as in (a).

IDENTIFICATION.

Place a drop of the alkaloidal solution on a clean glass slide; add a drop of reagent by means of a clean glass rod; and, without stirring or covering, examine under the

ALKALOID	REAGENT	CHARACTERISTICS OF PRECIPITATE
Cocaine	Platinic chloride	Delicate, feathery crystals that later become heavier in structure.
Codeine	Marme's	Silvery, circular masses that crystallize into dark rosettes of irregular outline.
	Wagner's	Heavy, reddish-brown precipitate that crystallizes very slowly in yellow blades extending in branches.
Morphine	Marme's	Silvery, gelatinous precipitate that crystallizes in dense masses of fine needles.
	Wagner's	Heavy, reddish-brown precipitate that crystallizes slowly in shining, red, overlapping plates extending in branches.
Strychnine	Platinic chloride	Crystals forming immediately in clusters and singly in small wedge-shaped needles that move about the field.
	Marme's	Silvery masses that form slowly in rosettes.

microscope, using low power. A magnification of 100-150 is suitable. Note the kind of crystals formed, and compare their characteristics with the descriptions given and then with a control.

SILVER PROTEINATES.

The following method for the determination of the acidity or alkalinity of silver proteinates was adopted as a tentative method:

Acidity or Alkalinity.—Tentative.

Dialyze 1 gram of the sample as directed under the official method for the detection of ionizable silver compounds and for the determination of ionizable silver¹ and titrate a portion of the clear solution representing 0.5 gram of the sample with either 0.02 *N* hydrochloric acid or 0.02 *N* sodium hydroxide solution, as required, using phenolphthalein indicator. Calculate acidity as percentage of hydrochloric acid and alkalinity as percentage of sodium hydroxide.

BIOASSAY OF DRUGS.

The following method for the assay of mydriatics and myotics was adopted as a tentative method:

MYDRIATICS AND MYOTICS.

Cat-eye Method.—Tentative.

APPARATUS.

(a) One cc. Mohr pipets, graduated in 0.1 cc., with slender tips that deliver exactly 0.05 cc. per drop.

(b) 100-watt, nitrogen-filled, electric lamps, or equally intense illumination.

ANIMALS.

Adult cats in good physical condition, weighing over 1500 grams, and accustomed to being handled.

PREPARATION OF SAMPLE.

Dissolve a representative number of tablets, or a sufficient quantity of powder, in approximately neutral distilled water, to make a solution containing 1 mg. of the alkaloid per cubic centimeter of solution. If the alkaloids themselves are taken, add the equivalent quantities of acid to convert the minto the corresponding salts. Add two drops of approximately 0.02 *N* acid per 50 cc. of solution.

For great accuracy, the results of chemical assay upon the sample should be followed in the preparation of solutions; when such accuracy is unnecessary, the declaration of strength on the label may be accepted as the basis for the preparation of the solution.

One drop of the respective concentrations of the following drugs have been found to be the minimum effective dose:

	MYDRIATICS.	mg. per liter.
Atropine	..	12
Hyoscyamine	..	4
Scopolamine	..	0 4
Homatropine	..	200
Cocaine	..	60
Euphthalmin.	..	50,000
Daturine	..	12
Duboisine	..	1.6
Ephedrine (alkaloid)	..	2,500
Ephedrine salt (or synthetic)	..	50,000
Pseudoephedrine (alkaloid)	..	2,500
Pseudoephedrine (salt)	..	80,000
	MYOTICS.	
Pilocarpine	..	25,000
Physostigmine (eserine)	..	10
Arecoline.	..	10,000

¹ *This Journal*, 1927, 10: 46.

DETERMINATION OF CAT'S THRESHOLD.

Place a cat about one foot from a 100-watt electric lamp, and determine the maximum contractility of its pupils under this condition. Drop 0.05 cc. of the freshly prepared standard mydriatic solution, obtained by diluting the 1 mg.-per-cc. solution, into the outer margin of one eye, leaving the other eye untreated as a control. Compress the inner canthus, while opening and closing the lids, until the fluid has apparently disappeared (10 to 30 seconds). Return cat to cage.

One and two hours after application (for atropine, 3 and 4 hours also), place cat under the same conditions, and note any differences in diameter between the pupils of the treated and the untreated eyes. (A satisfactory reaction is produced when the pupil of the treated eye is just perceptibly wider (0.5 to 1.0 mm.) than the pupil of the untreated eye.) Do not use the same eye for another assay for at least 24 hours.

If the concentrations given fail to produce a satisfactory reaction, repeat the test with a stronger or weaker solution until the minimum effective concentration is found. (This concentration may vary somewhat for different cats, but it is essentially constant for the same cat.)

BIOASSAY OF UNKNOWN SOLUTIONS.

Dilute the 1 mg.-per-cc. solution to be tested to the minimum effective concentration for the cats to be used, and drop 0.05 cc. of this dilution into one eye of the cat, following the same procedure as in the determination of the minimum effective concentration. Also prepare stronger and weaker solutions, and apply to one eye of each of the other cats used. Test various concentrations until one is obtained that produces satisfactory mydriasis of the same degree as the standard solution when tested on two or more cats.

To obtain the milligrams of alkaloid present in each cubic centimeter of the original solution, multiply the milligrams per cubic centimeter found to be the cat's minimum effective concentration by the dilution employed. Knowing that the original solution was made to contain 1 mg. of alkaloid per cubic centimeter, calculate the quantity of mydriatic present, and express as percentage of the total alkaloid.

XXXII. REFERENCE TABLES.

Table 10, Meissl's table for the determination of invert sugar in absence of sucrose (pp. 448-9), was deleted (first action).

EGGS AND EGG PRODUCTS.

The tentative method for the determination of the acidity of fat¹ was adopted as an official method (first action).

REPORT OF BOARD OF EDITORS.

One of the most vital and important questions that confront the Board of Editors and one that consequently interests all the members of the association is that of the support of *The Journal*. This support is naturally reflected in the number of subscriptions.

From 1923 to 1927, a period of four years, there has been a gradual decrease of domestic subscriptions due, partly, to cancellations, but

¹ *This Journal*, 1927, 10, 50.

mostly to non-payment of subscriptions. In reality, the association is better off financially through this prompter discontinuance of delinquent subscriptions.

There is one very encouraging fact, however, that this board would bring to the attention of members and that is the steady increase during the same period of the foreign subscriptions from 119 to 165, a gain of 46. It is felt that this has resulted from the far-sighted policy, inaugurated by the former boards, of an extensive foreign exchange list. Quite recently, a footing in Hungary was thus acquired, and it is hoped that a number of new subscriptions from this source may be reported next year. The latest available figures show a total of 845 subscribers.

At present the editorial office has a bound set of the first three volumes of *The Journal*. As it is felt that the association should have a permanent record of its proceedings, it is suggested that the seven additional volumes be bound as soon as the financial condition will warrant.

R. B. DEEMER.

W. F. HAND.

H. D. HASKINS.

Approved.

No report was made by the Committee on Quartz Plate Standardization and Normal Weight, but the following paper was presented by two members of the committee:

PRELIMINARY REPORT ON THE NORMAL WEIGHT OF SUCROSE FOR VENTZKE-SCALE SACCHARIMETERS.

By C. A. BROWNE (Bureau of Chemistry and Soils, Washington, D. C.)
and F. W. ZERBAN, (Sugar Trade Laboratory, New York, N. Y.).

Eight years ago, at the thirty-fifth annual meeting of the Association of Official Agricultural Chemists, a committee was appointed, consisting of Frederick Bates, Chairman, with C. A. Browne and F. W. Zerban as the other two members, to consider the questions of Quartz Plate Standardization and Normal Weight of Saccharimeters having the Ventzke scale. No meeting of this committee has thus far been called by Bates, and the present report is only in the nature of a preliminary statement upon some cooperative investigations which have been conducted during the past year under the direction of the other members of the committee.

At the Seventh Meeting of the International Commission for Uniform Methods of Sugar Analysis, which was held in New York in 1912,

Bates called attention to the fact that the polarization of the 26 grams normal weight of pure sucrose upon saccharimeters equipped with the Ventzke or German sugar scale gave a reading which was about 0.1° below the 100° point of the scale. The failure to hold any subsequent meetings, on account of the disturbances in international relations produced by the war, prevented the Commission from giving subsequent consideration to correcting any errors which may exist in the present 100° point of the saccharimeter scale. In 1916 Bates and Jackson of the Bureau of Standards, in Scientific Paper No. 268, published a detailed account of their experiments, from which they concluded that the polarization of 26 grams of sucrose under the prescribed conditions was 99.895° . This new value has since been adopted by the Bureau of Standards in the standardization of quartz control plates and saccharimeters.

In 1921 Stanek¹ published an article in which he gave a reading of only 99.81° for sugar purified by a double precipitation with alcohol.

In 1924 Kraisy and Traegel² of the German Institute for the Sugar Industry published the results of their extensive investigations upon this subject and the conclusion that the reading of 26 grams of pure sucrose, upon saccharimeters equipped with the Ventzke or German sugar scale, was 99.834° . This result, like that of Stanek, is considerably below the value of 99.895° reported by Bates and Jackson.

In view of the unfortunate lack of agreement upon this question, a series of cooperative experiments was initiated during the past year by the Bureau of Chemistry in Washington and the Sugar Trade Laboratory in New York City, in order to determine if confirmation could be obtained upon any of the results previously reported.

Briefly summarized, the investigations of the two laboratories consisted in polarizing exactly 26 grams of carefully purified sucrose, dissolved in distilled water to exactly 100 ml., at $20^{\circ}\text{C}.$, in tubes of 260 mm. length upon Schmidt and Haensch and Fric (Bates Model) saccharimeters, the scales of which had been graduated according to the Herzfeld-Schönrock value, namely, that 100 sugar degrees equal 34.657 circular degrees. The scales of the saccharimeters were constantly verified during the investigations by standardized quartz control plates, and all operations were carefully conducted under the prescribed conditions of temperature, light filtration, and other factors. The sucrose employed in the work was prepared in the Carbohydrate Laboratory of the Bureau of Chemistry by subjecting the purest obtainable grade of refined cane sugar to repeated crystallizations from water solutions that had been carefully concentrated in vacuo below $35^{\circ}\text{C}.$ The sugar was air dried and then sealed in glass bottles, from which samples were drawn for the

¹ *Z. Zuckerind. czechoslovak. Rep.*, 1921, 45: 417, 425.

² *Z. Ver. deut. Zucker Ind.*, 1924, 74: 193.

independent work of standardization at the two laboratories in New York and Washington.

The results obtained by C. A. Gamble and G. H. Hardin at the New York Sugar Trade Laboratory after making corrections for the slight amount of moisture and minute traces of ash in the sugar are given. Ten experiments (upon different weighed amounts of sucrose) of five observations each were conducted by each collaborator.

C. A. Gamble.—99.912 \pm 0.004 (99.881 — 99.938).

G. H. Hardin.—99.914 \pm 0.003 (99.885 — 99.935).

Average 99.913.

The results obtained by H. G. Hill of the Bureau of Chemistry as an average of eighteen experiments (upon different weighed amounts of sucrose) of five observations each and after making the necessary corrections for minute traces of moisture and ash were as follows:

H. G. Hill.—99.905 \pm 0.003 (99.875 — 99.934).

The general average of the results obtained at the Bureau of Chemistry and at the Sugar Trade Laboratory was 99.909, which is in close agreement with the result previously found by Bates and Jackson (99.895).

It is difficult to explain the rather wide difference between the higher results obtained by American observers and the lower results obtained by observers in Germany and Czechoslovakia. The results reported by Stanek in 1921 and by Kraisy and Traegel in 1924 were obtained upon sugar which had been purified by precipitation with alcohol, but it hardly seems possible that this could have affected the purity or polarization of the sucrose. Many grades of highly refined cane sugar sold in the United States show a greater polarization upon the German sugar scale than the average results reported by Stanek and by Kraisy and Traegel, and this would seem to indicate that their values are too low.

The question still remains as to what steps should be taken with respect to rectifying the present error in the German sugar scale, which is the type most generally used in the United States by sugar and food analysts. The average of the values obtained by Bates and Jackson in 1916 and by Gamble, Hardin, and Hill in 1927 is 99.902, which can be rounded out to the even decimal 99.9 without sensible error. This would require a normal weight of 26.026 grams of pure sucrose to give a reading of 100° upon the saccharimeters at present in most general use in the United States. In working with refined sugars, high grade sirups, and other products of high purity, where there are no counterbalancing plus errors due to the volume of lead precipitate and to other causes, it would be more accurate to employ a normal weight of 26.026 grams than the present weight of 26 grams. With products of low purity, however, where the counterbalancing plus errors may equal or exceed the minus

error due to the graduation of the scale, the introduction of a corrected normal weight or of a correction factor will increase instead of diminish the errors of observation. The plan to be recommended is to establish the true normal weight for the particular type of scale graduation and at the same time to correct for the lead precipitate and other errors, so that the uncertainties of counterbalancing disturbances may be eliminated.

The most satisfactory solution of the discrepancies observed in the standardization of the saccharimeter scale—the one proposed by Stanek in 1921—is for the investigators of the different countries to make an exchange in their preparations of sucrose and quartz control plates. The writers are inclined to believe that variations in the sensitiveness of the eyes of different observers to the slight optical disturbances produced by the unsymmetrical construction of the Lippich polarizer when a quartz wedge saccharimeter is used may explain a part of the trouble. The same observer obtains slightly different readings, depending upon whether the half prism of the Lippich polarizer is upon the right or left side of the field. Attempts are now being made to eliminate all errors due to personal equation by the construction of impersonal methods of polarization by means of sensitive photo-electric cells, but the mechanism has not been perfected to a sufficient degree of accuracy.

It is hoped that the incompleted details of the present cooperative investigations upon the normal weight of sucrose may be finished shortly so that the work may be written up for publication.

REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS.

The committee recommends the following definitions and interpretation of terms:

For Final Adoption as Official.

1. MEANING OF TERM "FINELY GROUND".

The term *finely ground* in the definition of basic phosphate slag shall refer to actual size of particles as determined by the use of standard sieves, as follows: seventy per cent (70%) or more should pass a 100-, and ninety per cent (90%) or more should pass a 50-mesh sieve.

2. NITRATE OF POTASH (COMMERCIAL POTASSIUM NITRATE).

Nitrate of potash is a salt containing not less than twelve per cent (12%) of nitrogen and forty-four per cent (44%) of potash (K_2O).

3. INTERPRETATION OF BRAND NAME TO INCLUDE THE ANALYSIS OR GRADE OF FERTILIZER.

The committee recommends and urges the practice of including the analysis or grade of fertilizer with the brand name, both by the manufacturer on sacks and in printed literature and by the control official in his reports and publications.

4. ACTIVITY OF WATER-INSOLUBLE NITROGEN IN MIXED FERTILIZERS.

The alkaline and neutral permanganate methods distinguish between the better and the poorer sources of water-insoluble nitrogen, and do not show the percentage availability of the material. The available nitrogen of any product can be measured only after carefully conducted vegetation experiments.

(a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to three-tenths of one per cent (0.3%) or more of the weight of the material. In the event of a total nitrogen exceeding the minimum guarantee, accompanied by a low activity of the insoluble nitrogen, the over-run may be taken into consideration in determining the classification of the water-insoluble nitrogen.

(b) The water-insoluble nitrogen in mixed fertilizers showing an activity below fifty per cent (50%) by the alkaline method and also below eighty per cent (80%) by the neutral method shall be classed as inferior. This necessitates the use of both methods before classifying as inferior.

Second Recommendation as Tentative.

1. MAXIMUM AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE POTASH IS CLAIMED AS SULFATE.

The *chlorine* in mixed fertilizers in which the potash is claimed as sulfate shall not exceed five-tenths of one per cent (0.5%) more than what is called for in the minimum potash content based on the definition for sulfate of potash as formulated by the committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.5.

2. DEFINITION OF PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALI SALTS CONTAINING POTASH.

These products are *not* potassium phosphate. They may be called non-acid phosphates with potash.

3. MURIATE OF POTASH (COMMERCIAL POTASSIUM CHLORIDE).

Muriate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) largely as chloride.

4. SULFATE OF POTASH (COMMERCIAL POTASSIUM SULFATE).

Sulfate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) largely as sulfate, and not more than two and one-half per cent (2.5%) of chlorine.

5. UNLEACHED WOOD ASHES.

Unleached wood ashes are ashes that result from burning unleached wood, that have had no part of their plant food removed, and that contain four per cent (4%) or more of water-soluble potash (K_2O).

6. NITRATE OF SODA (COMMERCIAL SODIUM NITRATE).

Nitrate of soda is a nitrogen salt containing not less than fifteen per cent (15%) of nitrogen, chiefly as sodium nitrate.

7. KAINIT.

Kainit is a potash salt containing potassium and sodium chlorides and sometimes sulfate of magnesia, with not less than twelve per cent (12%) of potash (K_2O).

8. DRIED BLOOD.

Dried blood is the collected blood of slaughtered animals, dried and ground and containing not less than twelve per cent (12%) of nitrogen in organic form.

9. GROUND STEAMED BONE.

Ground steamed bone is a product resulting from grinding animal bones that have been previously steamed under pressure.

10. GROUND RAW BONE.

Ground raw bone is a product resulting from drying and grinding animal bones that have not been previously steamed under pressure.

*Amended Tentative Interpretations and Definitions.***1. FERTILIZER GRADE.**

The *grade of a fertilizer* shall represent the minimum guarantee of its plant food expressed in terms of nitrogen, available phosphoric acid, and water-soluble potash.

*First Recommendation as Tentative.***1. TANKAGE.**

This term (without qualification) shall be restricted to meat and bone tankage derived from the rendered, dried, and ground by-products from the slaughter of animals, or from carcasses of animals that have died otherwise than by slaughter.

2. FISH TANKAGE, FISH SCRAP, DRY GROUND FISH.

This is the rendered, dried, and ground product derived from fish.

3. ACIDULATED FISH TANKAGE, FISH SCRAP, DRY GROUND FISH.

This is the rendered, dried, and ground product derived from fish and treated with sulfuric acid.

4. GARBAGE TANKAGE.

This is the rendered, dried, and ground product derived from waste household food materials.

5. CRUDE, INERT, OR SLOW-ACTING NITROGENOUS MATERIALS.

These are unprocessed organic substances relatively high in nitrogen but having a very low value as plant food and showing a low activity by both the alkaline and neutral permanganate methods (below 50% and 80%, respectively).

6. PROCESS TANKAGES.

These are made from crude inert nitrogenous materials by processing under steam pressure, with or without the use of acids, for the purpose of increasing the availability of the nitrogen.

These products shall not be called "tankages" without proper qualification.

7. HOOF AND HORN MEAL.

This is a product resulting from the processing, drying, and grinding of hoofs and horns.

8. SIGNIFICANCE OF THE WORDS BLOOD AND BONE AS THE BRAND NAME, OR PART OF THE BRAND NAME, OF A MIXED FERTILIZER.

The words *blood* and *bone* shall not be used as a part of the brand name of a mixed fertilizer unless all the organic nitrogen present is derived from blood and bone and all the phosphoric acid present is derived from bone or dissolved bone.

9. SUPERPHOSPHATE (ACID PHOSPHATE).

This is the ground product that results from mixing finely ground rock phosphate and crude sulfuric acid. As several grades are known to the trade, the grade should always be a prefix to the name. *Example:* 16% Superphosphate.

It is recommended that the use of the term "acid phosphate" be discontinued.

The following topics have been proposed for future consideration:

1. *Double Superphosphate.*
2. *Ammoniated Superphosphate.*
3. Significance of the words *Ammoniated Superphosphate* when used as a part of the brand name of a complete fertilizer.
4. Definition of product from wool carding establishments largely sheep manure. Seeds from plants and wool fiber.
5. Definition of *Activated Sewerage Sludge.*
6. Uniform order of terms for expressing the grade of a fertilizer.
7. *Agricultural Lime.*—As this term is indiscriminately applied not only to a great variety of products originating in the lime quarry but also to marl, shell lime, and refuse lime products from various industries, it is recommended that the use of the term be discontinued and that each specific lime product used as a soil amendment be defined.
8. *Quicklime, Burned Lime, Caustic Lime, Lump Lime, Unslaked Lime.*—This is commercial calcium oxide or a mixture of calcium oxide with varying smaller quantities of magnesium oxide resulting from heating suitable calcium containing minerals until substantially all the carbon dioxide has been eliminated. With a pure limestone the oxides should total 99 per cent.
9. *Hydrated or Slaked Lime.*—This is the product obtained by treating quick lime with sufficient water or steam to combine with its oxides. It usually contains about 65 per cent of calcium oxide or an equivalent of magnesium oxide.
10. *Air-Slaked Lime.*—This is the product resulting by exposure of caustic lime to the atmosphere, whereby it absorbs both moisture and carbon dioxide. It usually consists of a mixture of calcium oxide, calcium hydroxide, and calcium carbonate, or of these with smaller and varying quantities of the corresponding magnesium compounds.
11. *Ground Limestone.*—This is the product obtained by grinding calcitic or dolomitic limestone. Seventy-five per cent or more should pass a 100-mesh sieve. It should contain calcium and magnesium carbonates equivalent to not less than 45 per cent of calcium oxide or the mixed oxides of calcium and magnesium.
12. *Ground Shell Lime.*—This is the product obtained by grinding the shells of mollusks. Seventy-five per cent or more should pass a 100-mesh sieve and should contain calcium and magnesium carbonates equivalent to not less than 40 per cent of calcium oxide or the mixed oxides of calcium and magnesium.
13. *Marl, Ground Shell Marl.*—This is the product obtained by grinding natural deposits of shell marl. Seventy-five per cent or more should pass a 100-mesh sieve. It should contain calcium and magnesium carbonates equivalent to not less than 40 per cent of calcium oxide or the mixed oxides of calcium and magnesium.
14. *Waste Lime, By-Product Lime.*—This is any industrial waste or by-product containing calcium or calcium and magnesium in forms that will neutralize acids. It may be designated by the prefixation of the name of the industry or process by which it is produced, i. e., gas-house lime, tanners' lime, acetylene lime waste, lime-kiln ashes, etc.
15. *Calcium Sulfate, Gypsum.*—This is a neutral salt of calcium sulfate. It is accompanied by varying quantities of impurities including about 20 per cent of water. It does not neutralize acid solutions.

C. H. JONES.
J. W. KELLOGG.
G. S. FRAPS.

R. N. BRACKETT.
H. D. HASKINS.

Approved.

REPORT OF COMMITTEE ON REVISION OF METHODS OF SOIL ANALYSIS.

The First International Congress of Soil Science convened in Washington in June, 1927. The success of this meeting was of paramount importance to workers in soil chemistry in this country. Since most of those engaged in soil chemistry research were enlisted in the effort to insure the success of the Congress and devoted their available time and energy to that end, it has not been feasible to accomplish much toward further revision of methods during the past year. Moreover, it was realized that further revision would be facilitated after the presentation and discussion of the many methods proposed at the Congress. For these reasons the report of the committee should be an expression of continued interest and intent rather than accomplishment.

	W. H. MacINTIRE.	A. G. McCall.
	A. W. BLAIR.	J. S. McHARGUE.
Approved.	J. A. BIZZELL.	

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES.

Again the association is indebted to collaborators, associate referees, and referees for excellent contributions to the schedule of analytical methods. When it is remembered that all the contributors are already heavily taxed with their own special duties, the results secured are all the more gratifying. But the business of this association is unique, urgent, and serious, and if it is to hold the place of authority in the field which the scope of its work covers, the sacrifice of this added personal effort must continue to be made. What has been accomplished serves as a stimulus for the work that remains to be done.

Suggestions for improvements in the plan of work and in its operation have been made in these reports from time to time. To whatever extent the association may be falling short of the greatest accomplishment, however, the committee feels that the fault lies in the operation of the plan rather than in the plan itself. A working plan should be as simple as possible, and the present referee system appears to be as simple as it can well be made. As for the operation of the system, the committee feels that the observance of two points will increase efficiency more than anything else which can be suggested at the present time.

First, it is urged that work upon the recommendations for the ensuing year be begun at the earliest possible moment after the annual meeting of this association and while the stimulus of its sessions is still undi-

minated. It is fully appreciated that collaborative work in many, and perhaps all, cases, is necessarily subordinated to the individual's regular duties; nevertheless if associate referees will see to it that collaborative methods are in the hands of collaborators as early as possible, and will urge upon them the necessity for early completion of, and report upon, the work involved, an invaluable advantage will be gained.

The reason for this promptness is obvious and leads to the second point, viz., that those individuals and committees charged with the review of collaborative work and of recommendations adopt plans to secure more time for the consideration of reports and recommendations submitted. So far as referees and associate referees are concerned this question is solved insofar as the first suggestion is carried out. For committees it is urged that they meet sufficiently in advance of the opening session of the annual meeting to consider the business to come before them. Subcommittees A, B, and C should meet one day, and preferably two days, immediately before the opening of the regular sessions of the convention. No committee with much work to do can do it to advantage with the distractions that are bound to be encountered in the convention hall and its vicinity during sessions, and the evenings of convention days are largely devoted, and properly so, to social intercourse among attending members and delegates. With annual meetings beginning on Monday, as they have for many years, the earlier assembling of committees should not be difficult to arrange.

This committee urges the cooperation of all concerned in carrying out these two suggestions to the fullest extent possible in the coming year.

In accordance with a decision of the Executive Committee, three additional subjects are added this year to the scope of activities, and referees have been designated for these new or revived lines of work. The topics are paints, paint materials, and varnishes; caustic poisons; and beers, wines, and distilled liquors.

Reports of the heads of Subcommittees A, B, and C otherwise fully cover the work of this committee for the current year.

E. M. BAILEY.

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By J. W. KELLOGG (Department of Agriculture, Harrisburg, Pa.),
Chairman.

(To this committee are referred reports on the following general subjects: Waters, brine and salt; tanning materials and leathers; insecticides and fungicides; soils and liming materials; feeding stuffs; sugars and sugar products; fertilizers; plants; and paints, paint materials, and varnishes.)

WATERS, BRINE, AND SALT.

No report was submitted.

TANNING MATERIALS AND LEATHERS.

No report was submitted.

INSECTICIDES AND FUNGICIDES.

It is recommended—

(1) That the official methods for the determination of cyanogen and chlorine in sodium and potassium cyanides¹ be dropped (final action).

Approved.

(2) That the tentative method for the determination of cyanogen in sodium and potassium cyanides² be adopted as official (final action).

Approved.

(3) That Methods I and II for the determination of chlorine in sodium and potassium cyanides³ be adopted as official (final action).

Approved.

(4) That the tentative method for the determination of cyanogen in calcium cyanide⁴ be adopted as official (final action).

Approved.

(5) That Methods I and II for the determination of chlorine in calcium cyanide⁴ be adopted as official (final action).

Approved.

(6) That the official method for the determination of moisture in soap¹ be dropped (final action).

Approved.

(7) That the xylene distillation method for the determination of water in soap⁵ be adopted as official (final action).

Approved.

(8) That the methods for the determination of water, total oil, and ash in mineral-oil-soap emulsions⁶ be adopted as official (final action).

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 65.

² *This Journal*, 1927, 10: 27.

³ *Ibid.*, 28.

⁴ *Ibid.*, 29.

⁵ *Ibid.*, 1926, 9: 28, 29.

(9) That Method II for the determination of soap in mineral-oil-soap emulsions¹ be adopted as official, with the following note appended: "Error will result in this method if the apparent molar weight of the acids varies appreciably from that of oleic acid" (final action).

Approved.

(10) That in the tentative method for the determination of unsulfonated residue in mineral oils², the third sentence, which reads as follows: "In lieu of measuring, determine the specific gravity of the oil and weigh the equivalent of 5 cc. into the bottle" be amended to read as follows: "If greater accuracy is desired, the measured charge may be weighed and its exact volume calculated from the weight and specific gravity of the oil".

Approved.

FLUORINE COMPOUNDS.

It is recommended that the methods suggested by the associate referee be studied further.

Approved.

SOILS AND LIMING MATERIALS.

REACTION VALUE OF SOILS.

A report on the Reaction Value of Acid Soils was given, but no report was submitted by the Associate Referee on Alkaline Soils.

It is recommended that this subject be referred to the Committee on Revision of Soil Analysis for consideration of the recommendations made last year³.

Approved.

LIMING MATERIALS.

It is recommended—

(1) That the sugar method for the determination of the caustic value of lime, as described by the associate referee, be adopted as an official method (first action).

Approved.

(2) That the tentative methods, I and II, for the determination of calcium oxide in burnt lime and hydrated lime⁴ be deleted from *Methods of Analysis*.

Approved.

(3) That no report of the investigations of the solubility of soil potassium having been received, this subject be continued.

Approved.

¹ *This Journal*, 1926, 9: 28.

² *Ibid.*, 1927, 10: 30.

³ *Ibid.*, 62.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 36.

LESS COMMON METALS IN SOILS.

No report was submitted.

It is recommended that the associate referee continue the study of methods for the determination of manganese and the less common metals in soils.

Approved.

FEEDING STUFFS.**STOCK FEED ADULTERATION.**

It is recommended that the method for the detection of dried buttermilk in feeding stuffs, as reported by the associate referee, be adopted as tentative (see p. 36).

Approved.

MINERAL MIXED FEEDS.

It is recommended—

(1) That the proposed method for the determination of lime in mineral feeds and the method for the determination of iodine in mineral feeds, as reported by the associate referee, be further studied.

Approved.

(2) That methods for the determination of iodine in organic materials be studied.

Approved.

DETERMINATION OF MOISTURE.

It is recommended—

(1) That the Bidwell-Sterling method¹ for the determination of water, approved for first action as official last year, be not presented for final action at this time.

Approved.

(2) That the methods for the determination of moisture outlined by the associate referee be further studied to include such materials as molasses feed and linseed meal.

SUGARS AND SUGAR PRODUCTS.**HONEY.**

It is recommended that the procedure of Auerbach and Bodlander, recommended by the associate referee for the detection of the adulteration of honey with commercial invert sugar, as well as the polariscopic method at elevated temperatures and Jackson's modification of Nyn's method of selective determination of fructose, recommended by the referee, be subjects of collaborative studies.

Approved.

¹ *This Journal*, 1926, 9: 30.

MAPLE PRODUCTS.

No report was submitted.

The committee wishes to correct an error in the report published last year on page 63 of Vol. X, No. 1, to the effect that no report on maple products was submitted. The report of H. M. Lancaster, the Associate Referee on Maple Products, was published in Vol. X, No. 2, on page 180.

STARCH CONVERSION PRODUCTS.

No associate referee was appointed.

DRYING, DENSIMETRIC AND REFRACTOMETRIC METHODS.

No report was submitted.

It is recommended that the recommendations of 1926¹ be repeated.

POLARISCOPIC METHODS.

It is recommended—

(1) That, as suggested by the associate referee, a further study be made of the four inversion methods reported in his investigation, and that they be applied to mixtures of pure sugar, and pure amids and amino acids.

Approved.

(2) That the isolation in the pure state of the amids and amino acids occurring in cane blackstrap molasses be attempted.

Approved.

CHEMICAL METHODS FOR REDUCING SUGARS.

It is recommended—

(1) That, as recommended by the associate referee, Meissl's determination for invert sugar be discarded and that the first three lines on page 195, *Methods of Analysis*, A. O. A. C., 1925, and Table 10, pages 448 and 449, be deleted (first action).

Approved.

(2) That Scale's volumetric method² and Benedict's method³ for the determination of small quantities of sugar be studied.

Approved.

(3) That Benedict's modification⁴ of the Folin and Wu method⁵ for minute quantities of sucrose be studied.

Approved.

(4) That the associate referee's modification of Nyn's method⁶ for the determination of fructose in the presence of other reducing sugars be subjected to further study.

Approved.

¹ *This Journal*, 1927, 10: 64.

² *J. Ind. Eng. Chem.*, 1919, 11: 747.

³ *J. Biol. Chem.*, 1909, 5: 485.

⁴ *Ibid.*, 1926, 68: 759.

⁵ *Ibid.*, 1920, 41: 367.

⁶ *Bull. assoc. école sup. brasserie Louvain*, 1925, 25: 63-76; *C. A.*, 1925, 19: 1236.

(5) That methods for the determination of extremely small quantities of reducing sugars in the presence of large quantities of sucrose, as recommended by the referee, also be studied.

Approved.

FERTILIZERS.

PHOSPHORIC ACID.

It is recommended—

(1) That the calcium chloride method for preparing ammonium citrate¹ be eliminated from the methods (final action).

Approved.

(2) That the present phase of the collaborative study of the gravimetric determination of phosphoric acid be discontinued.

Approved.

(3) That the words "Nearly neutralize with strong hydrochloric acid", line 12, in the gravimetric determination of phosphoric acid (I, 7)² be changed to read, "Neutralize with strong hydrochloric acid, using litmus paper or bromthymol blue as indicator" (first action).

Approved.

(4) That the direction to "burn first at a low heat and then ignite intensely until white or grayish white", line 17, in the gravimetric determination of phosphoric acid (I, 7)² be changed to read "burn first at a low heat and ignite to constant weight preferably in an electric furnace, at 950°-1000°C." (first action).

Approved.

(5) That the words "dilute to 1 liter" in the second alternative method [I 5 (c) (2)]³ for the preparation of magnesia mixture be changed to read "proceed as in (1)" (final action).

Approved.

(6) That a third alternative method for the preparation of magnesia mixture [I 5 (c)]³ be worded as follows (first action):

(3) "Dissolve 55 grams of crystallized magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in water, add 140 grams of ammonium chloride, and dilute to 870 cc. Add strong ammonium hydroxide to each required portion of the solution just before using at the rate of 15 cc. per 100 cc. of solution".

Approved.

NITROGEN.

It is recommended—

(1) That the absolute or cupric oxide method for the determination of nitrogen⁴ be removed from the methods for fertilizers (final action).

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 4, Sec. 13 (2).

² *Ibid.*, 3.

³ *Ibid.*, 2.

⁴ *Ibid.*, 9.

(2) That the Jones method¹ for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide and (or) urea (see p. 32) be adopted as a tentative method.

Approved.

(3) That the associate referee be instructed to compare results obtained by the method suggested by B. F. Robertson, with the results obtained by the Jones method for the determination of nitrate nitrogen.

Approved.

(4) That the official zinc iron method for the determination of nitric and ammoniacal nitrogen² be discarded (first action).

Approved.

(5) That the official reduced iron method for the determination of nitric and ammoniacal nitrogen² be marked, "Applicable only in the absence of cyanamide and urea" (first action).

Approved.

NITROGEN ACTIVITY METHODS.

It is recommended—

(1) That in Par. 38, line 3³, the word "water-insoluble" be inserted before the word "nitrogen", so as to have the phrase read, "making a correction for the water-insoluble nitrogen of the filter, if necessary" (first action).

Approved.

(2) That in Par. 40³, the directions for the preparation of alkaline permanganate solution be changed to read as follows: "Dissolve 25 grams of potassium permanganate in hot water and, separately, 150 grams of sodium hydroxide in cold water; combine the solutions when cold; and dilute to 1 liter. Discard any permanganate solutions that have become green in color" (first action).

Approved.

(3) That in Par. 41 (a)⁴, after line 3, the following statement be added: "When it is found necessary to use 4 or more grams of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed" (first action).

Approved.

(4) That in Par. 42, line 3⁴, after the sentence, "transfer from the filter to a 500-600 cc. Kjeldahl distillation flask", the following phrase be added: "loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers" (first action).

Approved.

¹ *Ind. Eng. Chem.*, 1927, 19: 269.

² *Methods of Analysis*, A. O. A. C., 1925, 11.

³ *Ibid.*, 12.

⁴ *Ibid.*, 13.

(5) That in Par. 42, line 4¹, in place of "a piece of paraffin the size of a pea", the following phrase be substituted, "a drop of mineral lubricating oil weighing not more than 50 mg." (first action).

Approved.

(6) That in Par. 42, line 5, the sentence, "Connect with, etc." be changed to read as follows: "Connect with an upright condenser to the lower end of which has been attached a 100 cc. graduated cylinder containing standard acid, and so arranged as to receive the distillate below the surface of the acid or otherwise so trapped as to prevent loss of ammonia fumes" (first action).

Approved.

(7) That in Par. 42, line 7, the words "at least" be deleted from the sentence, "Digest slowly with a very low flame, for at least 30 minutes, below distillation point", and that the phrase "below distillation point" be changed to read "barely below distillation point" (first action).

Approved.

(8) That in Par. 42, line 9, the direction to "distil until 95 cc. of distillate is obtained", be changed to read as follows: "Distil 95 cc. in 60 minutes (plus or minus 5 minutes), controlling the distillation so that approximately 24 cc. of distillate is obtained in each 15 minute period. Conduct the first part of the distillation over a bare flame but use wire gauze 10 minutes before completion to avoid breaking the flask". Also that before the direction to "Titrate with standard alkali" there be inserted the added direction to "transfer the distillate to an Erlenmeyer flask or to a beaker" (first action).

Approved.

(9) That to Par. 42, the following directions be added: "If the active water-insoluble nitrogen is found to be less than 55 per cent of the total water-insoluble organic nitrogen present, it is recommended that a second portion of the sample be prepared as directed under 41 (a). Dry the residue below 80°C., transfer from the filter to a Kjeldahl flask as directed above, and determine the nitrogen as directed under 19 or 22. Recalculate the percentage of active water-insoluble nitrogen on the basis of the quantity of water-insoluble nitrogen thus found" (first action).

Approved.

(10) That the study of the nitrogen activity methods be continued with a view to discovering:

- a. The effect of different degrees of fineness in material ground to pass a 1 mm. sieve.
- b. The appropriateness of stricter standards for the alkaline permanganate solution used.

¹ *Method of Analysis*, A. O. A. C., 1925, 13.

- c. The application of the method to mixtures containing uric acid.
Approved.

It is recommended—

(1) That the study of the use of calcium carbonate in preparing the solution for the determination of potash be continued.

Approved.

(2) That the method for the determination of chlorine in fertilizers given in the report of the associate referee be adopted as tentative (see page 34).

Approved.

PLANTS.

PREPARATION OF PLANT MATERIAL FOR ANALYSIS.

It is recommended that this work be continued.

Approved.

LESS COMMON METALS IN PLANTS.

No report was submitted.

It is recommended that this work be continued.

Approved.

TOTAL CHLORINE IN PLANTS.

It is recommended—

(1) That collaborative work be undertaken on a method for the determination of inorganic chloride in plant materials.

Approved.

(2) That further work include investigations to ascertain whether or not organic chlorine can be determined by ashing methods.

Approved.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By A. G. MURRAY (Food, Drug and Insecticide Administration, Department of Agriculture, Washington, D. C.), *Chairman*.

(To this committee are referred reports on the following general subjects: Specific gravity and alcohol, spices and other condiments, naval stores, drugs; and beer, wines, and distilled liquors.)

SPECIFIC GRAVITY AND ALCOHOL.

It is recommended that the associate referee ascertain the opinion of not less than fifty members of the association working in different laboratories on the procedure for the determination of alcohol and the ar-

rangement of tables with a view to consideration of the desirability of any changes in the present procedure and tables.

Approved.

SPICES AND OTHER CONDIMENTS.

No report was submitted. The committee recommends a repetition of the recommendation of 1925 relative to salad dressing¹.

Approved.

NAVAL STORES.

TURPENTINE.

No formal report was presented. The committee recommends further study of the subject.

Approved.

DRUGS.

ALCOHOL IN DRUGS.

It is recommended that the methods studied by the referee during the past year, with the exception of that for the determination of alcohol in the presence of iodine, be again submitted to collaborative investigation with tentative adoption in view at the next meeting of the association.

Approved.

ARSENICALS.

It is recommended—

(1) That the method proposed by the referee for the determination of arsenic in sodium cacodylate last year² (see p. 48) be made official (first action).

Approved.

(2) That the method proposed by the referee for the determination of arsenic in iron methylarsenates be made tentative.

Approved.

COCAINE.

It is recommended that the two methods for the determination of cocaine described by the referee, modified to specify the use of peroxide-free ether (see p. 49), be made tentative and that no further work be done on this subject at this time.

Approved.

CHAULMOOGRA OIL.

It is recommended that no further work be done on this subject.

Approved.

CRUDE DRUGS.

No report was submitted.

¹ *This Journal*, 1925, 8: 284.

² *Ibid.*, 1926, 9: 287.

CHLOROFORM AND CARBON TETRACHLORIDE.

It is recommended that further collaborative study be given to methods for the determination of chloroform and carbon tetrachloride in drugs.

Approved.

IPECAC ALKALOIDS.

It is recommended that the Palkin-Murray-Watkins automatic extraction method¹ and the Palkin-Watkins hand extraction method² for the determination of ipecac alkaloids (see p. 50) be made tentative, as recommended by the associate referee.

Approved.

RADIOACTIVITY IN DRUGS AND WATER.

No report was submitted. The committee recommends the continuation of the work as outlined in Recommendation 2 of 1924³.

Approved.

LAXATIVES AND BITTER TONICS.

No report was submitted. It is recommended that work be continued as outlined in 1924³.

Approved.

MERCURIALS.

It is recommended that the method for the determination of calomel in calomel tablets described in the report of the referee as Method 4 (the iodine method) be made tentative (see p. 51).

Approved.

PYRAMIDON.

It is recommended—

(1) That the method for the determination of pyramidon proposed by the referee (see p. 51) be made tentative in lieu of the two present tentative methods⁴.

Approved.

(2) That no further work be done on this subject.

Approved.

MICROCHEMICAL METHODS FOR ALKALOIDS.

It is recommended—

(1) That the methods proposed by the referee for the identification of morphine, codeine, cocaine, and strychnine (see p. 52) be made tentative.

Approved.

¹ *Ind. Eng. Chem.*, 1925, 17: 612.

² *J. Am. Pharm. Assoc.*, 1924, 13: 694.

³ *This Journal*, 1925, 8: 267.

⁴ *Ibid.*, 546.

(2) That further studies for the identification of other alkaloids by microchemical methods be made.

Approved.

SILVER PROTEINATES.

It is recommended—

(1) That the titration method for the determination of acidity or alkalinity proposed by the referee be made tentative (see p. 53).

Approved.

(2) That no further work be done on this subject.

Approved.

TERPIN HYDRATE.

It is recommended that the method for the determination of terpin hydrate described by the referee be studied collaboratively and that further methods for the determination of this drug be sought.

SANTONIN.

No report was submitted. It is recommended that collaborative study be given to the methods outlined by the referee in 1925¹.

Approved.

ETHER.

It is recommended that the method described by the referee for the determination of ether be studied collaboratively.

Approved.

BIOASSAY OF DRUGS.

It is recommended that the cat-eye method for the determination of mydriatics and myotics (see p. 53) be made tentative.

Approved.

FLUIDEXTRACT OF GINGER.

It is recommended that an associate referee be appointed to study methods for the determination of the volatile oil and its constants and the non-volatile ether-soluble extractive and its constants in fluidextract of ginger.

Approved.

GENERAL.

It is also recommended that the following new subjects be studied, and that an associate referee be appointed for each subject: Bromides-chlorides, ephedra, menthol, oil of chenopodium, pilocarpine in tablets, *sabadilla* (assay of), and *thymol*.

Approved.

¹ *This Journal*, 1926, 9: 326.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS
OF REFEREES.

By H. A. LEPPER¹ (Food, Drug and Insecticide Administration, Washington, D. C.), *Acting Chairman*.

(To this committee are referred reports on the following general subjects: Dairy products, fats and oils, baking powders and baking chemicals, eggs and egg products, food preservatives, coloring matters in foods, metals in foods, fruits and fruit products, canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products, gelatin, cacao products, cereal foods.)

DAIRY PRODUCTS.

BUTTER.

It is recommended—

(1) That the method for sampling print butter given in the 1926 report² and recommended by the associate referee for adoption as official, be not adopted as official at this time, in order to give the referee an opportunity to conduct a study with a view to expanding the method to include directions as to the number of subdivisions necessary to represent a given lot of print butter.

Approved.

(2) That the method for sampling tub butter² be further studied with the object of expanding this method to include the number of tubs to be sampled to represent a given lot of tub butter.

Approved.

(3) That the mixing modification be removed from the present official method for preparing a sample of butter³, as recommended by the associate referee, by deletion of the words "or mix", line 2 (first action).

Approved.

(4) That a study be made of the shaking modification of the present official method as given in the associate referee's report, with a view to determining the best conditions for the preparation of the sample of butter and describing these conditions in order to eliminate any uncertainties.

Approved.

(5) That the method of analysis proposed by the associate referee be made a tentative method and that it be studied further, because it is realized that it is useful as a rapid method for sorting out samples.

Approved.

(6) That the associate referee make a definite recommendation next year in regard to the recommendation made in 1926⁴ on the suitability of containers for butter samples.

Approved.

¹ Member of Subcommittee C, of which C. F. Whitney is Chairman. E. M. Bailey is the other member of this committee. J. O. Clarke assisted in the work of the committee.

² *This Journal*, 1927, 10: 292.

³ *Methods of Analysis*, A. O. A. C., 1925, 276.

⁴ *This Journal*, 1927, 10: 72.

CHEESE.

It is recommended—

(1) That the methods for the determination of tartaric and citric acids in cheese, as given in the report of the associate referee, be adopted as tentative methods (see p. 40) and also be studied collaboratively.

Approved.

(2) That further work be done on the phosphorus pentoxide: calcium oxide ratios of processed cheese.

Approved.

(3) The committee repeats the recommendation of 1926¹ that methods for the detection of preservatives, coloring matters, emulsifying agents, or other added substances in cheese be further studied.

MALTED MILK.

It is recommended—

(1) That the tentative method for the determination of moisture in malted milk² be dropped.

Approved.

(2) That the official method for the determination of moisture in cheese³ be adopted as a tentative method for the determination of moisture in malted milk after the following changes have been made: Use 1-1½ grams of sample instead of 2-3 grams and dry for 5 hours instead of for 4 hours.

Approved.

(3) That the present tentative method for the determination of fat in dried milk⁴ be adopted as a tentative method for the determination of fat in malted milk after the following changes have been made: Omit the directions for the addition of ammonia by substituting for the sentence, "Add 9 cc. more of water and 1 cc. of ammonia", the sentence, "Add 10 cc. of water".

Approved.

(4) That methods for the determination of various sugars in mixtures containing malted milk and dried milk be studied.

Approved.

(5) That further work be done on the microscopic identification of malted milk.

Approved.

(6) The committee also recommends that the methods now in effect for malted milk be included in *Methods of Analysis*, Chapter XIX, "Dairy Products", under the sub-title "Dried Milks and Malted Milks".

Approved.

¹ *This Journal*, 1927, 10: 72.

² *Methods of Analysis*, A. O. A. C., 1925, 275.

³ *This Journal*, 1926, 9: 44.

⁴ *Ibid.*, 1925, 8: 482.

DRIED MILK.

It is recommended—

(1) That the official method for the determination of moisture in cheese¹ be adopted as tentative for the determination of moisture in dried milk after the following change has been made: Use 1-1½ grams of sample instead of 2-3 grams and dry for 5 hours instead of for 4 hours.

Approved.

(2) That methods for the determination of various sugars in mixtures containing dried milk and malted milk be studied.

Approved.

(3) The committee also recommends that the methods now in effect for dried milk be included in *Methods of Analysis*, Chapter XIX, "Dairy Products", under the sub-title "Dried Milks and Malted Milks".

Approved.

ICE CREAM.

It is recommended—

(1) That owing to the uncertainty of the nature of the work contemplated in the following recommendation of the associate referee, he discuss plans for future work with the incoming General Referee on Dairy Products: That the proposed method for ash in ice cream be submitted to further collaborative study in connection with its value as a basis for the calculation of milk solids not fat, and further, that a collaborative study be made of the average composition of normal milk in an effort to produce all available data on the subject, as well as to establish ratios as a basis for the calculation of milk solids not fat in ice cream.

Approved.

(2) That the modified Ferris method for gelatin in ice cream² be submitted to collaborative study.

Approved.

(3) That the Kjeldahl-Gunning-Arnold method for the determination of total nitrogen be adopted as a tentative method in the analysis of ice cream in view of the fact that this is a recognized official method. The directions are to provide for the use of 4-5 gram sample as stated by the associate referee in his report.

Approved.

(4) The committee also recommends that methods for the determination of milk protein in ice cream be further studied.

MILK PROTEINS.

It is recommended that further study be given to methods for the determination of milk proteins.

Approved.

¹ *This Journal*, 1926, 9: 44.

² *Ibid.*, 1927, 10: 317.

QUALITATIVE TESTS.

It is recommended that the method for the detection of gelatin be studied collaboratively.

Approved.

FATS AND OILS.

It is recommended—

(1) That the André-Cook method for the determination of acetyl value¹ be made official (final action).

Approved.

(2) That no further study be made during the coming year of the Thomas-Yu and the modified Bellier methods for the approximate determination of peanut oil in the presence of olive oil.

Approved.

(3) That the study of the lead-salt-ether method for the determination of saturated and unsaturated fatty acids be continued.

Approved.

(4) That the study of the Twitchell-lead-salt-alcohol method for the determination of saturated fatty acids be discontinued.

Approved.

(5) That the method for the "Cold Test" as applied to salad oil be studied.

Approved.

(6) That the present official method for the determination of acetyl value² be dropped (final action).

Approved.

BAKING POWDER.

It is recommended—

(1) That the modified gasometric method for the determination of total carbon dioxide³ be adopted as official (final action).

Approved.

(2) That the following sentence: "1 to 3 drops of caprylic alcohol may be added to the baking powder in the decomposition flask to prevent foaming", be added to the directions for the gasometric determination of residual carbon dioxide⁴, as amended⁵, and that the method be made official (final action).

Approved.

(3) That study be continued on the separation and determination of the different forms of phosphates used as baking acids.

Approved.

(4) That consideration be given to methods for the determination of alumina.

Approved.

¹ *This Journal*, 1927, 10: 35.

² *Methods of Analysis*, A. O. A. C., 1925, 293.

³ *This Journal*, 1927, 10: 36.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 306.

EGGS AND EGG PRODUCTS.

It is recommended that the method for the determination of the acidity of the fat, as described in the 1926 report of the associate referee, be adopted as official (first action). This method was tentatively adopted in 1926¹.

Approved.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, AND ASH.

It is recommended—

(1) That further study, accompanied by collaborative work if possible, be made of the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol and unsaponifiable matter.

Approved.

(2) That collaborative study be made of the methods for ashing, fat by acid hydrolysis, lipoids, and lipid phosphoric acid.

Approved.

DETECTION OF DECOMPOSITION.

It is recommended that the following methods for the detection of decomposition be studied: Acid-soluble phosphoric acid, ammonia nitrogen, and reducing substances as dextrose.

Approved.

TOTAL SOLIDS.

It is recommended that in recognition of the report on the study of methods for moisture in eggs by the referee, as recommended in 1926, further collaborative study be made of the 98°C. vacuum oven method, with a view to its adoption as official.

Approved.

FOOD PRESERVATIVES.

It is recommended—

(1) That the search for a more accurate method for the determination of benzoic acid (or of sodium benzoate) in food products be continued.

Approved.

(2) That in this work as many classes of food products as is possible be included in the study.

Approved.

(3) That the application of the process of sublimation to the separation and purification of saccharine to be determined in food products be made the subject of study.

Approved.

(4) That an effort be made to formulate a satisfactory method for the detection of hydrogen peroxide added to food products.

Approved.

¹ *This Journal*, 1927, 10: 50, 74, 411.

COLORING MATTERS IN FOODS.

It is recommended—

(1) That further study be devoted to the method described in the referee's report in regard to the quantitative separation and estimation of amaranth and tartrazine and that more experimental data be collected.

Approved.

(2) That work be undertaken to obtain a chemical method for the separation and quantitative estimation of fast green F C F from light green S F yellowish and guinea green B and other permitted dyes.

Approved.

(3) That the recommendation of 1925 that additional work be done on separating yellow A B and yellow O B from other oil-soluble dyes be repeated.

Approved.

METALS IN FOODS.

It is recommended—

(1) That methods listed by the referee and others as suitable for the determination of arsenic be further studied.

Approved.

(2) That tentative method I¹ for the determination of lead be so modified as to be applicable to foods and that the resulting modification and the thiocyanate modification of this tentative method be studied collaboratively.

Approved.

(3) The committee repeats the recommendation of 1926 that a study of methods for the determination of copper and zinc be continued.

Approved.

ZINC IN DRIED EGGS.

No report has been received from the Associate Referee on this subject since 1924².

The committee recommends that this study be included in the general studies on zinc by the General Referee on Metals in Foods.

Approved.

FRUITS AND FRUIT PRODUCTS.

It is recommended—

(1) That the recommendation made in 1925³ that a further comparison be made of the refractometric and official vacuum methods⁴ for the determination of solids in solutions containing sucrose and organic acids be repeated.

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 90.

² *This Journal*, 1925, 8: 621.

³ *Ibid.*, 1926, 9: 85.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 179, 210.

(2) That the recommendation made in 1925¹ that consideration be given to all methods now included by reference in the chapter on Fruits and Fruit Products be repeated and that the referee submit to the association preliminary directions, if these are necessary, to make the methods definite and complete.

Approved.

(3) That the suggestion of the referee that the report of the Committee on Nomenclature of Pectin of the Agriculture Food Division of the American Chemical Society² be considered by this association and the definition endorsed be not approved.

The committee feels that provision has already been made by this association for matters pertaining to definitions and standards for foods and materials entering into the composition of food through its representatives on the Food Standards Committee.

Approved.

ASH IN FRUIT PRODUCTS.

It is recommended—

(1) That the determination of larger quantities of manganese be studied and that collaborative work be done on the determination of the major bases in plant ash—potash, calcium, magnesium, and manganese.

Approved.

(2) That methods for the determination of iron and aluminum in plant ash be undertaken.

Approved.

(3) That, as suggested by the referee, the title of Sec. 12, Chapter XIV, page 211 of *Methods of Analysis*, be changed from "Chlorides" to "Chlorine in the Ash".

This is a change in title only of an official method and more exactly designates the determination made.

Approved.

(4) That study be made of the application to fruits of the associate referee's proposed procedure for chlorine in plant ash.

Approved.

FRUIT ACIDS.

It is recommended that further study be made of methods for the determination of malic acid and other fruit acids.

Approved.

CANNED FOODS.

No report was submitted.

¹ *This Journal*, 1926, 9: 85.

² *Proc. Am. Chem. Soc.*, 1927, p. 37.

It is recommended—

(1) That the recommendation made in 1925¹ regarding the study of methods for the detection of spoilage in canned foods be repeated.

Approved.

(2) That as recommended in 1925¹ the Referee on Canned Foods submit a method for distinguishing between sweet corn and field corn to collaborative study.

Approved.

VINEGARS.

It is recommended—

(1) That methods for the determination of total and soluble ash involving the use of substances to assist in the formation of a porous ash material, such as sugar, be further studied.

Approved.

(2) That the official methods for the determination of soluble and insoluble phosphoric acid² be further studied.

Approved.

(5) That further study be made of methods for the determination of glycerol, sulfates, and polarization.

Approved.

MEAT AND MEAT PRODUCTS.

It is recommended that the following section be deleted from the official method for total nitrogen in meats³ (first action): "In the Kjeldahl and Gunning methods digest with sulfuric acid for at least 4 hours; in the Kjeldahl-Gunning-Arnold method, for 2 hours after the mixture has become clear". The method will then read as follows: "Proceed as directed on p. 7, 19, or p. 8, 22, or 24, using about 2 grams of the fresh sample". This change in the official method suggested by the referee substitutes the recognized official methods for the determination of nitrogen.

Approved.

SEPARATION OF MEAT PROTEINS.

No report was submitted.

FLAVORS AND NON-ALCOHOLIC BEVERAGES.

It is recommended—

(1) That the steam distillation method for the determination of oils of lemon, orange, and lime in corn and cottonseed oils and in mineral oil, described in the report of the referee this year, be adopted as official (first action). This method was studied in 1925⁴ and this year (see p. 45).

Approved.

¹ *This Journal*, 1926, 9: 86.

² *Methods of Analysis*, A. O. A. C., 1925, 325.

³ *Ibid.*, 237.

⁴ *This Journal*, 1926, 9: 450.

(2) That study of the method referred to in Recommendation I be continued with a view to extending its use to other non-alcoholic flavors.

Approved.

(3) That the colorimetric method for the determination of small quantities of anthranilic acid ester, described in the report of the referee this year, be adopted as official (first action). (See p. 46.)

Approved.

(4) That the gravimetric method for the determination of large quantities of anthranilic acid ester, described in the report of the referee this year, be adopted as official (first action). (See p. 47.)

Approved.

GELATIN

No report was submitted.

It is recommended that the following recommendations of 1926¹ be repeated:

(1) That further study be made of the method of preparation of the sample by ashing as compared with that by hydrolysis.

Approved.

(2) That the determination of copper be studied for the purpose of developing a more satisfactory method than is now available.

Approved.

(3) That the precipitation of zinc in formic acid solution be considered in further studies of methods for the determination of this metal.

Approved.

CACAO PRODUCTS.

It is recommended—

(1) That the method for cacao shell² be designated as a tentative method and that for the sake of uniformity the methods for coloring matters, Section 28, be also so designated.

The method for cacao shell was adopted as tentative in 1921³.

Approved.

(2) That the official method for the determination of fat in cacao products be dropped (final action).

Approved.

(3) That methods for the determination of milk solids and sucrose in cacao products be studied.

Approved.

MICROSCOPICAL METHODS.

No report was submitted.

¹ *This Journal*, 1927, 10: 77.

² *Methods of Analysis*, A. O. A. C., 1925, 347.

³ *This Journal*, 1922, 6: 150.

CRUDE FIBER.

It is recommended—

(1) That modification of the methods for the determination of crude fiber applicable to milk cacao products be studied.

Approved.

(2) That the method for crude fiber proposed by the associate referee be studied further and that collaborative work be done.

Approved.

CACAO BUTTER.

It is recommended—

(1) That the method described by the associate referee for the detection of coconut oil and palm kernel oil in cacao butter and in fat from milk chocolate be adopted as tentative (see p. 45).

Approved.

(2) That study be continued on methods for the detection of foreign fats in cacao fat and in the fat of cacao products in general.

Approved.

CEREAL FOODS.

FLOUR.

It is recommended—

(1) That the tentative method for sampling flour¹ be adopted as official (first action).

Approved.

(2) That the air-oven method for the determination of total solids and moisture (indirect method) in flour² be adopted as official (final action).

Approved.

(3) That the associate referee continue studies on rapid methods for the determination of ash in flour, omitting, however, further consideration of the alundum method.

Approved.

(4) That the associate referee study the glycerol-alcohol modification of the official method for the determination of ash in flour³, with a view to its adoption as a tentative method.

Approved.

(5) That the associate referee study the nature and kind of losses occurring when ash is fused.

Approved.

¹ *This Journal*, 1926, 9: 39.

² *Ibid.*, 40.

³ *Methods of Analysis*, A. O. A. C., 1925, 225.

(6) That the acid hydrolysis method for the determination of fat in flour¹ be adopted as an official method (final action).

Approved.

(7) That the F. A. C. method for the determination of unsaponifiable matter in fats and oils, as modified and adopted at the 1926 meeting², be adopted as tentative for flour and subjected to further collaborative study. (This determination is made on the lipoids extracted as directed in the method for the determination of lipoids³.)

Approved.

(8) That further collaborative studies be conducted involving only the barium hydroxide method for glutenin⁴, with special precautions to insure that all collaborators use the same reagents (which should be of unquestioned purity) and the same procedure in minute details.

Approved.

(9) That the tentative method for the determination of the hydrogen-ion concentration of flour⁵, amended as recommended by the associate referee by the following addition to the last sentence, "using electrodes and a potentiometric set-up which have been checked through the use of a buffer solution of known hydrogen-ion concentration", be adopted as an official method (first action).

Approved.

(10) That the associate referee study the possible use of the quinhydrone and antimony electrodes in the determination of the hydrogen-ion concentration of flour.

Approved.

(11) That further study of the method for the determination of gluten in flour be discontinued until such time as a definite need exists for its standardization and modification.

Approved.

(12) That the designation "Gluten" for the tentative method on page 227 of the 1925 edition of *Methods of Analysis* be changed to read "Crude Gluten".

Approved.

(13) That Par. 16, p. 227, of the 1925 edition of *Methods of Analysis* be amended to read "Quantitative Method.— Tentative (results are approximate)".

Approved.

(14) That the study of methods for the determination of the diastatic value of flour be continued.

Approved.

¹ *This Journal*, 1926, 9: 41.

² *Ibid.*, 45; 1927, 10: 35.

³ *Ibid.*, 1926, 9: 40.

⁴ *Cereal Chem.*, 1927, 4: 129.

⁵ *This Journal*, 1927, 10: 33.

(15) That further study be made on the determination of chlorine in chlorine bleached flour.

Approved.

(16) That the Rask method for starch¹, as modified by the associate referee, be adopted as tentative and subjected to further collaborative study (see p. 37).

Approved.

(17) That the modification of the diastase method for starch, as suggested by Hartmann and Hillig², be studied.

Approved.

(18) That the factors for the conversion of the percentages of nitrogen into terms of protein in wheat, wheat bran, wheat endosperm, and wheat embryo, as suggested by Jones³, be adopted.

Approved.

(19) That the official vacuum method for moisture in flour⁴ be dropped (final action).

Approved.

BAKED CEREAL PRODUCTS.

It is recommended—

(1) That collaborative study of the tentative method⁵ for the preparation of a sample of bread⁶ be continued.

Approved.

(2) That the tentative method for the determination of total solids of an entire loaf of bread⁶ be further studied.

Approved.

(3) That the tentative method for the determination of total solids of the air-dried ground sample⁶ be further studied.

Approved.

(4) That studies of the 130°C. air-oven⁶ and other rapid methods for the determination of total solids in an entire loaf of bread be continued.

Approved.

(5) That comparative studies of the methods for the determination of lipoids (as directed for alimentary pastes⁶) and of fat in bread be continued.

Approved.

(6) That the study of methods for the carrying out of experimental baking tests be continued.

Approved.

¹ *This Journal*, 1927, 10: 108.

² *Ibid.*, 1926, 9: 482.

³ *Cereal Chem.*, 1926, 3: 194.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 238.

⁵ *This Journal*, 1926, 9: 42.

⁶ *Ibid.*, 40.

(7) That consideration be given to the development of methods for the determination of milk solids in milk bread.

Approved.

(8) That consideration be given to the development of methods for the determination of rye flour in rye bread.

Approved.

ALIMENTARY PASTES.

It is recommended—

(1) That the tentative method for taking and preparing a sample of alimentary paste for analysis¹ be studied collaboratively.

Approved.

(2) That the tentative method for the determination of total solids and moisture (indirect method¹) be studied collaboratively.

Approved.

(3) That the study of the air-oven method for the determination of total solids in flour² as adopted for this determination in alimentary pastes be continued.

Approved.

(4) That the tentative acid hydrolysis method for the determination of fat in alimentary pastes, with the slight change suggested by the associate referee, be adopted as official (first action). (See p. 38.)

Approved.

(5) That the tentative method for the determination of lipoids and lipid phosphoric acid (P_2O_5) in flour² be adopted as official for these determinations in alimentary pastes (first action).

Approved.

(6) That the F. A. C. method for unsaponifiable matter in fats and oils, as recommended for tentative adoption for flour², be tentatively adopted for the determination of unsaponifiable matter in alimentary pastes.

Approved.

(7) That the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol⁴ be further studied.

Approved.

¹ *This Journal*, 1926, 9: 43.

² *Ibid.*, 40.

³ *Ibid.*, 45; 1927, 10: 33.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 232.

REPORT OF THE REPRESENTATIVES OF THE A. O. A. C. ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL¹.

It is the purpose of the representatives to report such investigations as are being actively conducted under the auspices of the Institute, so that the members of this association may be in a position to learn through its president, W. C. O'Kane, Durham, N. H., where and by whom the work is being done.

There follows a list of the organizations which are taking advantage of the plan of the Institute, in accordance with which they are meeting the expenses incident to research on the named products, to ascertain their direct or indirect value in combating pests and troubles which interfere with the normal output of farm products.

American Association of Nurserymen: Crown gall, callus formation.

Bayer Company: (1) Penetration of seed coats by seed-borne parasites, (2) thallium and mercury compounds as insecticides and rodenticides, (3) control of southern field crop diseases.

California Spray-Chemical Company: "Volck" for combating external parasites of cattle and sheep.

Kay Research Laboratories, Inc., Nyack, N. Y.: Oxidized oils, e. g., "alcohol", for combating insects and animal parasites.

Koppers Company, Pittsburgh: Colloidal sulfur.

Nichols Copper Company: A fundamental study of the value of copper compounds in agriculture.

Quaker Oats Company: Furfural derivatives for seed treatment.

Standard Oil Company of Indiana: (1) Emulsified foliage spraying oils, as "dendrol", (2) cattle sprays.

Standard Oil Company of New Jersey: (1) "Flit" for eliminating household and live-stock insects, borers, ground animals, etc., (2) pyrethrum production, (3) horticultural sprays, (4) relation of size of particles to efficiency.

By consultation with those who are conducting these researches, those who are particularly interested may get early information concerning the lines of investigation which are under way.

The following papers on the investigations of the Institute have been published during the year:

Lambert, E. B., Rodenhiser, H. A., and Flor, H. H.—The effectiveness of various fungicides in controlling the covered smuts of small grains. Crop Protection Digest No. 10, and Phytopathology, Vol. 16.

Melhus, I. E.—A summary of the results of the cooperative investigation of crown gall in relation to apple nursery stock. Crop Protection Digest No. 11.

Nelson, Franklin C.—The penetration of a contact oil spray into the breathing system of an insect. Crop Protection Digest No. 12.

Flor, H. H.—Fungicidal activity of furfural. Crop Protection Digest No. 13, and Iowa State Journal of Science, Vol. 1.

Stewart, M. A.—A means of control of the European hen flea (*Ceratophyllus gallinae* Schrank). Crop Protection Digest No. 14, and Journal of Economic Entomology, Vol. 20.

Any member of the A. O. A. C. who has not joined the Institute may become a scientific member by conferring with Paul Moore, Secretary of Crop Protection Institute, National Research Council, Washington, D. C., and by paying one dollar annually.

An idea of the breadth of the Institute may be gained by one of its purposes, namely: to further cooperation between scientific workers and the producers of chemicals; the manufacturers of insecticides, fungicides, and other similar materials; the manufacturers of appliances required for their use; and the manufacturers, growers, packers, and shippers of the foregoing and of plant, animal, and other products.

BURT L. HARTWELL.

H. J. PATTERSON.

Approved.

REPORT OF THE SECRETARY-TREASURER.

The work of the Secretary-Treasurer for this year has not varied materially from other years. Perhaps a larger number of inquiries regarding the work and membership of the association has been handled, and this is especially true in regard to foreign countries.

One of our most active workers, Julius Hortvet, has passed on since the last meeting. A biographical sketch and tribute was written by W. W. Randall and published in Volume X, No. 3. It is with regret also that it is necessary to note the death of Harry Snyder, a past president of this association, and of Frederick B. Power and E. H. Golaz, who have been prominently identified with the activities of the association. Sketches outlining the careers of these members have been prepared by the Committee on Necrology, which was appointed at a recent meeting of the executive committee. The members of this committee are W. W. Randall, C. A. Browne, and H. C. Lythgoe. Suitable resolutions will be presented by the Committee on Resolutions.

METHODS OF ANALYSIS.

The 3,031 copies of the 1925 edition of *Methods of Analysis*, completed in June, 1925, at a cost of \$8,969.13, were exhausted in October, 1927. This bill was paid during 1925 and 1926. The second printing of 2,145 copies was made and delivered in part on August 18, 1927, at a cost of \$2,734.88. Copies are being mailed at the average rate of 50 per month, which rate should insure an ample supply for three years, or until another edition. The bill for this second printing and the one for Volume 10,

No. 4, which has not been received, are the only bills unpaid, aside from a few small amounts incurred in connection with this meeting.

JOURNAL.

It is regretted that no increase can be reported in the subscription list and resources of the *Journal*. Last year the foreign subscriptions increased somewhat, but a proportionate increase was not maintained this year. A few requests for complete sets of the *Journal* continue to be received, and these are filled at regular subscription rates as fast as copies of Volume I can be obtained. If any of the members of the association have these copies and wish to dispose of them, please notify the secretary. The present subscription list is 845, including 32 exchanges, complimentary, and advertisers' copies. The number should be at least 1,000, and it is suggested that each member constitute himself a committee of one to see that there is an increase.

RESIGNATIONS AND APPOINTMENTS.

The following resignations were received: R. W. Balcom, chairman of the Board of Editors, and A. J. Patten, member of the Board of Editors, member of Committee on Editing Methods of Analysis, and General Referee on Plants.

H. D. Haskins resigned from the Chairmanship of the Committee on Definitions of Terms and Interpretation of Results on Fertilizers, but remained as a member of the committee, and C. H. Jones was appointed as chairman.

R. B. Deemer was appointed Chairman of the Board of Editors, and F. C. Blanck and H. R. Kraybill were named as members of this board, but no appointments were made of General Referees on Plants and Dairy Products and to fill the vacancy on the Committee on Editing Methods of Analysis. Just before this meeting the secretary received word that owing to illness Deemer would be unable to serve as Chairman of the Board of Editors. The Executive Committee requested Blanck to act in this capacity temporarily.

C. A. Browne, W. W. Skinner, and W. H. MacIntire were appointed to represent the association at the First International Congress of Soil Science, and J. C. Munch and L. E. Warren, to represent the association at the National Conference on Pharmaceutical Research.

The Executive Committee also designated C. A. Browne, editor, and W. W. Skinner, associate editor, of the revised edition of "Principles and Practice of Agricultural Analyses," and nominated Guy G. Frary to serve on the permanent committee to cooperate with other committees on food definitions and recommended his appointment to the president.

The committee voted to include in the next issue of *Methods of Analysis* revised chapters on wines, beers, and distilled liquors. The

committee also recommended the appointment of a permanent committee on necrology.

The following tabulations show the financial condition of the *Journal* and *Methods of Analysis*, as well as dues, for the year ending October 1, 1927.

W. W. SKINNER.

FINANCIAL REPORT OF THE SECRETARY-TREASURER FROM OCTOBER 1, 1926, TO OCTOBER 15, 1927.

RECEIPTS.

1926			
Oct.	1	Bank balance	\$1,056.71
		1926 dues received too late for inclusion in 1926 report, 11 at \$5.00	55.00
Dec.	14	A. O. A. C. share of cost of programs for Joint Meetings	28.89
		1927 dues from institutional members, 61 at \$5.00 . . .	305.00
Total			\$1,445.60

DISBURSEMENTS.

		Amount	Check No.
1926			
Oct.	26	Marian E. Lapp, 1926 meeting expenses	\$7.75 52
		Montgomery Mutual Bldg. & Loan Asso. Bond	1,000.00 53
Nov.	2	Association Dairy, Food and Drug Officials, share for badges	20.34 54
Nov.	9	R. W. Balcom, refund for amount credited to dues instead of to <i>Journal</i> account	5.00 55
Dec.	6	R. W. Balcom, refund for amount credited to dues instead of to <i>Journal</i> account	5.00 56
Dec.	14	Industrial Printing Co., bills of Oct. 22 and Nov. 13 (2)	72.45 57
1927			
Mar.	2	Industrial Printing Co., bill of 8-24-27, 1200 programs, 1926 meeting	34.50 58
May	2	Geo. C. Schaffer, flowers for J. Hortvet	21.72 59
Sept.	22	Industrial Printing Co., bill of 9-13-27, 1200 programs, 1927 meeting	41.50 60
Total		\$1,208.26	
Oct.	15	Bank balance	237.34
Total		\$1,445.60	

FINANCIAL REPORT ON PUBLICATIONS FROM

RECEIPTS.

<i>Methods of Analysis.</i>			
Number	Price each	Total	
44	\$5.50	\$242.00	
356	5.00	1,780.00	
99	4.40	435.60	
172	4.00	688.00	
		<u>\$3,145.60</u>	
Plus gain on exchange.....		.15	
Total receipts.....			\$3,145.75
<i>Journal Subscriptions.</i>			
Number	Price each	Total	
2	\$8.25	\$16.50	
10	7.50	75.00	
6	6.60	39.60	
4	6.00	24.00	
64	5.50	352.00	
416	5.00	2,080.00	
95	4.40	418.00	
229	4.00	916.00	
6	4.50	27.00	
6	2.50	15.00	
15	1.50	22.50	
2	1.25	2.50	
3	1.20	3.60	
		<u>\$3,991.70</u>	
Plus payment on one subscription from bankruptcy sale, Puyallup & Sumner Fruit Growers Canning Co.....		.18	
Plus gain on exchange.....		.34	
Total.....			3,992.22
<i>Advertisements.</i>			
Number	Price each	Total	
10	\$25.00	\$250.00	
3	15.00	45.00	
Total.....			295.00
<i>Miscellaneous.</i>			
3 years' subscription to Journal of Biological Chemistry for Brazilian subscriber		\$10.50	10.50
<i>Reprints.</i>			
Smith, Aifend, Mitchell.....		\$6.54	
S. Aifend.....		4.96	
C. H. Bailey.....		4.96	
E. M. Bailey.....		7.39	
L. C. Mitchell.....		4.27	
H. Runkel.....		2.15	
University of Nebraska.....		4.92	
J. J. T. Graham.....		1.00	
School of Hygiene and Public Health, Johns Hopkins University.....		15.50	
O. S. Rask.....		15.20	
Armour Fertilizer Works.....		12.38	
New York Sugar Trade Laboratory.....		2.11	
New Jersey Agricultural Experiment Station (Prince).....		10.46	
Division of Feed, Minnesota Dairy and Food Department (Halvorson).....		2.68	
McCandless Laboratory (McCandless).....		2.14	
H. C. Waterman.....		1.00	
Total.....			\$97.66
Total for Methods, Journal, Ads, Reprints, and Miscellaneous.....			\$7,541.13
Plus balance in bank turned over to W. W. Skinner from R. W. Balcom upon his resignation as Chairman of the Board of Editors.....			1,031.40
			\$8,572.53
Minus re-deposited checks.....			50.40
			\$8,522.13
Total bank balance of October 1, 1926.....			545.88
Total.....			\$9,068.01

OCTOBER 1, 1926, TO OCTOBER 1, 1927.

DISBURSEMENTS.

		Amount	Check No.
1926			
Oct. 23	Estelle L. Milne, office expenses	\$50.00	190
Oct. 27	Baker & Co., Inc., back number of <i>Journal</i>	1.25	191
Nov. 13	Franklin Square Subscription Agency, refund on subscrip- tion	1.20	192
Nov. 15	Industrial Printing Co., bill of 5-29-26	1,021.44	193
Nov. 24	P. L. Ricker, stencils	22.08	194
Nov. 27	Cash, mailing <i>Journals</i> and office expenses	50.00	195
Nov. 29	Purnell Stationery Co., refund on subscription	16.00	196
Dec. 7	Postmaster, Washington, D. C., mailing <i>Journals</i>	25.00	197
Dec. 21	Postmaster, Washington, D. C., box rent, quarter ending 3-31-26	2.00	198
1927			
Jan. 12	San Francisco News Co., refund on subscription	4.50	199
Jan. 14	Estelle L. Milne, service	50.00	200
Jan. 15	Industrial Printing Co., bills of 9-21-26, 11-10, 13-26	43.72	201
Jan. 15	Industrial Printing Co., bill of 8-31-26	1,050.65	202
Jan. 15	G. P. Walton, back numbers of <i>Journal</i>	4 00	203
Jan. 25	Estelle L. Milne, office expenses	50.00	204
Feb. 17	Industrial Printing Co., bill of 11-17-26	316.25	205
Feb. 19	L. E. Warren, back numbers of <i>Journal</i>	4.38	206
Mar. 2	Carleton Book Store, refund on duplicate payment for <i>Methods</i>	4.00	207
Mar. 8	Braun Corp., discount allowed on <i>Methods</i>	12.00	208
Mar. 16	Industrial Printing Co., on account, bill of 12-17-26	600.00	209
Mar. 19	The Colonial Printery, bill of 3-18-27	15.65	210
Mar. 21	A. Domenech, Spain, refund on <i>Journal</i> subscriptions	2.20	211
Mar. 21	Postmaster, Washington, D. C., box rent, quarter ending 6-30-27	2.00	212
Mar. 25	W. E. Hillier, back numbers of <i>Journal</i>	4.00	213
Apr. 2	Industrial Printing Co., balance on bill of 12-17-26	774.52	214
Apr. 11	W. E. Hillier, back numbers of <i>Journal</i>	4.00	215
Apr. 18	Industrial Printing Co., bills of 1-11-27, 3-28-27, and (2) 4-8-27	147.65	216
May 13	Estelle L. Milne, mailing <i>Journals</i> and office expenses	50.00	217
May 20	Industrial Printing Co., bill of 2-28-27	1,195.50	218
June 20	Postmaster, Washington, D. C., box rent, quarter ending 9-30-27	2 00	219
June 30	W. W. Skinner, change of account from R. W. Balcom	1,031.40	220
July 7	Estelle L. McCoy, office expenses	50 00	221
July 14	J. Biological Chemistry, for Brazilian subscriber	10 50	222
July 16	A. Cliffe, Toronto, back numbers of <i>Journal</i>	10.00	223
July 19	Underwood and Underwood, picture of Hortvet	5.00	224
July 27	Industrial Printing Co., bills of 6-17, 22-27	1,187.63	225
July 29	J. J. Betton, bond for Mrs. Malcolm McCoy	2.50	226
Sept. 8	Estelle L. McCoy, office expenses	50.00	227
Sept. 21	Postmaster, Washington, D. C., box rent, quarter ending 12-31-27	2.00	228
	Plus bank balance, October 1, 1927	1,192.99	
Total		\$9,068.01	

REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS.

The committee begs to submit the following report upon the proceedings of the Joint Committee on Definitions and Standards for the past year.

Julius Hortvet, for many years a representative of this association upon the Joint Committee on Definitions and Standards, died on April 7th, 1927. An appreciation of him, and of his service to this body and to food chemistry in general, has already been placed in the records of the association, but it is fitting that his colleagues should emphasize his loyalty and his invaluable contributions to the work for which this association chose him.

Two meetings of the Joint Committee have been held, one during the week of November 29, 1926, and the other during the week of March 28, 1927.

At the November meeting the subject of alimentary pastes, which had been under consideration for several sessions, was reconsidered and finally adopted. The following schedule was approved by the Department of Agriculture and promulgated in Food Inspection Decision 206, dated February, 1927:

Alimentary pastes are the shaped and dried doughs prepared from semolina, from farina, from wheat flour, or from a mixture of any two or of all of these, with or without salt, and with one or more of the following: water, egg, egg-yolk, milk, a milk product.

An alimentary paste contains not more than thirteen per cent (13%) of moisture, as determined by the vacuum method.

Plain alimentary pastes are alimentary pastes made without egg or egg-yolk, or so made that the content of the solids of egg and/or of egg-yolk is, upon a moisture-free basis, less than five and one-half per cent (5.5%) by weight.

Egg alimentary pastes are alimentary pastes which contain, upon a moisture-free basis, not less than five and one-half per cent (5.5%) by weight of the solids of egg and/or of egg-yolk.

Noodles, egg noodles, are a form of egg alimentary paste which, in the course of its preparation, has been rolled or pressed into sheets or ribbons, with or without subsequent cutting or shaping.

Water noodles are a form of plain alimentary paste which, in the course of its preparation, has been rolled or pressed into sheets or ribbons, with or without subsequent cutting or shaping.

Macaroni, spaghetti, vermicelli, are plain alimentary pastes, distinguished by their characteristic shapes.

Semolina macaroni, semolina spaghetti, semolina vermicelli, are plain alimentary pastes in the preparation of which semolina is the only farinaceous ingredient used, and are distinguished by their characteristic shapes.

The committee spent some time in consideration of a corn product which is rather widely designated in trade as "cream meal". It appears that this product has been developed in modern milling practice and that it differs in some important respects from old-fashioned, or stone-

ground, corn meal. A conference was held at which certain millers appeared, and briefs were submitted by others interested. Because of conflicting testimony at hand, and because of numerous protests against a proposed definition submitted by a group of the milling industry, the committee was unable to arrive at any satisfactory conclusion as to the identity of this product, and the subject is held for further study which may properly include other additions to, or revisions of, the present grain products schedule so far as it concerns corn products.

The term "buttermilk", unqualified, according to the present definition applies to a product which is obtained in the process of churning milk or cream, sweet or sour. A similar product has long been recognized in trade, however, and which is made by souring skimmed, or partially skimmed, milk by means of lactic acid-producing bacteria. The committee adopted a tentative definition and standard for this newer product. At the March meeting the tentative definition was reconsidered. Manufacturers and others interested were present, and their comments and criticisms were heard. After further consideration a final definition and standard was adopted, which was later approved by the Secretary of Agriculture and promulgated in Food Inspection Decision 210, dated April, 1927. The definition is as follows:

Cultured buttermilk is the product obtained by souring pasteurized skimmed, or partially skimmed, milk by means of a suitable culture of lactic bacteria. It contains not less than eight and five-tenths per cent (8.5%) of milk solids not fat.

The question of a definition and standard for dried buttermilk, concentrated buttermilk, was discussed, and the matter was referred to Hortvet for investigation.

There was some discussion of the subject of *ice cream*, but no revision of the tentative definition and standard already adopted by the committee was made. A definition for so-called *Maraschino cherries* was considered at the request of importers, but after some discussion the proposal did not appear to warrant further consideration. Likewise a request for a definition of "*smoked salt*" was dismissed after due consideration and the hearing of those interested.

In response to resolutions by the Association of American Dairy, Food and Drug Officials, a definition and standard for so-called "*process cheese*" was considered. Some information had already been gained by the committee at a hearing of manufacturers and control officials last year, and further testimony was given by Congressman Voigt, representing the Wisconsin Cheese Manufacturers Association. The committee formulated a tentative definition and standard for the product. The National Cheese Makers Federation later requested a hearing on this subject, and representatives of that organization, headed by Senator Lenroot, were heard at the March meeting. More data were secured,

and a revision of the tentative definition was drafted by the committee, but no affirmative action was taken.

Rice is defined in Circular 136 as the hulled, or hulled and polished grain of *Oryza sativa*. The term "rice", unqualified, has come to be commonly used to designate the article generally sold as "rice" and which is, in fact, polished rice. To avoid the confusion which has arisen in the minds of consumers and to properly distinguish between brown rice and the polished grain, the old definition of rice was amplified. The following definition has been approved by the Department of Agriculture and promulgated as Food Inspection Decision 208, dated February, 1927:

Rice is the hulled, or hulled and polished, grain of Oryza sativa.

(a) *Brown rice* is the hulled, unpolished grain.

(b) *Polished rice*, "rice" is the hulled grain from which the bran or pericarp has been removed by scouring and rubbing.

The definition of sweetened condensed milk was revised with respect to a minor question of phraseology. The revision was approved by the Department of Agriculture and promulgated as Food Inspection Decision 207, dated February, 1927. The revised form is as follows:

Sweetened condensed milk is the product resulting from the evaporation of a considerable portion of the water from milk to which sugar (sucrose) has been added. It contains not less than twenty-eight per cent (28%) of total milk solids, and not less than eight per cent (8%) of milk fat.

The Macaroni Manufacturers Association requested a consideration of definitions for semolina and farina. Representatives of that association and others interested were heard, and the committee gave some time to discussion of the question. A tentative definition for each product was drafted as a basis for further consideration and to secure a fuller expression of opinion both from manufacturers and control officials.

In response to a desire on the part of certain State food officials for a classification of strained tomato products, this question was considered at some length by the committee. No hearing was held, but experts from the Department of Agriculture were called in conference, and a tentative schedule of classification was drafted. Careful study of this subject will be necessary in order to classify these products in such a way as to be helpful to enforcement officials and at the same time not to conflict with established classifications for tariff purposes now in force for imported tomato products.

C. D. HOWARD.
E. M. BAILEY.

Approved.

No report was given by the Committee on Sampling.

REPORT OF THE COMMITTEE TO CONSIDER THE ADVISABILITY OF STUDYING METHODS FOR THE ANALYSIS OF PAINT.

The last report of this committee was published in the *Journal*¹. As a result of a study of the report, the Executive Committee has made several suggestions which the committee is glad to adopt and to embody in its report this year.

Without reviewing the considerations which led to the suggestion that the association undertake the systematic study of the methods of paint analysis, the committee now desires to amend its former report as follows:

(1) That the concluding paragraphs (1), (2), and (3), as they appear on page 110 of volume IX of the *Journal* be deleted. In the place of the procedure outlined in these paragraphs, the committee now recommends merely the study of methods of paint analysis and suggests the appointment of a general referee and of associate referees, in accordance with the usual practices of the association.

W. F. HAND.
J. W. KELLOGG.
W. T. PEARCE.

Approved.

¹ *This Journal*, 1926, 9: 107.

THIRD DAY.
WEDNESDAY—AFTERNOON SESSION

REPORT OF COMMITTEE ON BIBLIOGRAPHY.

This is a brief report of progress. Of the six subjects that have been assigned for revision, one has been completed and submitted to the committee. The other five are in the process of completion. It is planned to have all of these in shape for the 1928 meeting. There are also several subjects which have not been assigned, but arrangements have been made for some of them and it is expected that active work will be begun this year.

	W. W. SKINNER.	H. D. HASKINS.
	G. S. FRAPS.	W. W. RANDALL.
Approved.	F. P. VEITCH.	

REPORT OF COMMITTEE ON CONSTITUTION AND BY-LAWS.

The Chairman of the Committee on Constitution and By-Laws made one attempt to call a meeting of the committee early in the year while in Washington, but two members of the committee were ill and one was out of the city. It was not possible for the members to get together before this meeting, and work of this sort cannot be done successfully by correspondence. Therefore, the chairman is indebted to W. W. Skinner, a member of this committee, W. H. MacIntire and O. Schreiner, members of the Executive Committee, and to C. A. Browne, a past president of the association, for their work in preparing the tentative revised draft, which was submitted to the chairman of the committee and approved. It is this draft that has been considered today and has now been adopted. The chairman desires to express again his appreciation of the valuable services rendered by the above mentioned members of the association.

	B. B. ROSS.	R. B. DEEMER.
	E. M. BAILEY.	W. W. SKINNER.
Approved.	F. P. VEITCH.	

The principal changes made in the Constitution were the addition of new subjects for study, namely, caustic poisons and paints, paint materials and varnishes, and the provision to include in the membership all those persons that are working along the lines included in the objects of the association. The other changes were made to clarify the phraseology. A copy of the former Constitution will be found on page 586 of Volume 3. The Constitution as revised this year is as follows:

CONSTITUTION.

ARTICLE I.

Name and Object.

This association shall be known as the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS OF NORTH AMERICA¹.

The objects of the association shall be—

1. To secure, devise, test, and adopt uniform and accurate methods for the analysis of fertilizers, soils, foods, feeding stuffs, dairy products, insecticides and fungicides, and other materials relating to agricultural pursuits; also medicinal products; caustic poisons; paints, paint materials, and varnishes;
2. To secure uniformity in the statement of analytical results;
3. To conduct, promote, and encourage research in chemistry in its relation to agriculture;
4. To afford opportunity for the discussion of matters of interest to agricultural chemists.

ARTICLE II.

Membership.

ACTIVE MEMBERS.

Chemists connected with the following institutions of North America¹ shall alone be eligible *ex officio* to active membership:

1. The United States Department of Agriculture;
2. Any national, state, or provincial experiment station, college, or body engaged in research in agricultural chemistry;
3. Any national, state, or provincial institution or body charged with official control of any of the materials named in Article I.

ASSOCIATE MEMBERS.

Chemists connected with municipal laboratories in North America charged with control of any of the materials or subjects named in Article I are eligible *ex officio* to associate membership.

Chemists engaged in research in agricultural chemistry who are not eligible to active membership and active members of the association who lose their right to such membership by retiring from the positions indicated above as requisite for eligibility to active membership may be elected to associate membership upon recommendation of the Executive Committee.

HONORARY MEMBERS.

Upon recommendation of the Executive Committee, persons may be elected to honorary membership by the two-thirds vote of those present at any regular meeting of the association.

ARTICLE III.

Officers and Committees.

The officers of the association shall consist of a president, a vice-president, and a secretary who shall also act as treasurer.

These officers shall be elected annually from and by the active members and they shall perform the usual duties of their respective positions. These officers, the immediate past president, and three other active members to be elected by the association shall

¹ NOTE: The term North America is intended to include the United States and its colonial possessions, Canada, Cuba, the British West Indies, Haiti, San Domingo, Mexico, and the republics south of Mexico as far as the Panama Canal. This excludes the French West Indian Islands of Martinique and Guadeloupe.

constitute the Executive Committee. In case of the inability or disability of any one of the additional members of the Executive Committee, any past president may be designated to serve in his stead. The special duties of the officers of the association shall be further defined, when necessary, by the Executive Committee. With the concurrence of the Executive Committee, the president shall appoint a chairman and a committee of nine other members, which shall be designated as a Committee on Recommendations of Referees, one-third of the membership of which shall be appointed at intervals of two years to serve six years, the chairman to be appointed annually. The chairman shall divide the nine members into subcommittees (A, B, and C) and shall assign to each subcommittee the reports and subjects which it shall consider. At the annual meeting, upon the recommendation of the Committee on Recommendations of Referees, the president shall appoint from among the active or associate members of the association a referee and associate referees for each of the subjects to be considered by the association. It shall be the duty of these referees—

1. To direct and conduct research on the methods and subjects assigned to them;
2. To prepare and distribute samples and standard reagents to members of the association and others;
3. To present at the annual meeting the results of the work done and recommendations for methods to be based thereon; and
4. To direct and encourage general discussion at the meetings.

ARTICLE IV.

Meetings.

The annual meeting of the association shall be held at such place as shall be decided by the association, and at such time as shall be decided by the Executive Committee. Announcement thereof shall be made, if possible, three months prior to the time of said meeting. Special meetings shall be called by the Executive Committee when in its judgment it may be necessary.

ARTICLE V.

Changes in Constitution.

All proposed changes or amendments to this constitution shall be presented in writing and read in full to the association not later than the second day of the regular annual meeting, shall be referred to the Executive Committee, and after a report from this committee may be adopted as the first order of business on the third day by a vote of three-fourths of the active members present.

BY-LAWS.

1. Any amendment to these by-laws or changes therein may be proposed in the same manner and adopted by the same procedure as amendments to the constitution, but only a two-thirds vote of the active members present shall be required for their adoption.

2. These by-laws or any portion of them may be suspended at any regular meeting of the association without previous notice, by a vote of three-fourths of the active members present.

3. Only one qualified active member of a department, college, experiment station, board, or other institution shall be entitled to vote on general questions before the whole association. At the discretion of the Chair, any institutional vote upon which there does not seem to be adequate representation may be conducted by letter ballot.

4. In voting upon questions involving methods of analysis, definitions, nomenclature, and laws or regulations relating to materials mentioned in Article I of the constitution, each of the said institutions shall be entitled to vote only upon questions relating to those materials over which said institution exercises official control.

5. No method shall be adopted as official nor shall any official method be amended until such method or amendment has been recommended for adoption by the appropriate referee at two annual meetings.

6. No changes shall be made in the methods of analysis used in official inspection until an opportunity shall have been given all active members having charge of the particular inspection affected to test the proposed changes.

7. No method shall be adopted as tentative nor shall a tentative method be amended until such method or amendment has been recommended for adoption by the appropriate referee and published in the proceedings of the association.

8. When any officer ceases to be eligible for membership in the association, his position shall be considered vacant, and a successor may be appointed by the Executive Committee to continue in office until the next regular meeting. Should any referee or associate referee resign or cease to be eligible for membership in the association, his office shall be considered vacant and a successor shall be appointed as prescribed in Article III of the constitution. Should a vacancy occur in the Executive Committee, such vacancy may be filled by the action of the other members.

9. Chemists connected with commercial firms or institutions and others interested in the objects of the association who are not eligible to either active or associate membership may attend its meetings, take part in the discussions, and, if permission is secured from the Executive Committee, may present papers.

10. Each department, college, experiment station, board, or other institution entitled to representation in the association shall contribute annually \$5.00 prior to the first of January following the regular annual meeting.

11. A Board of Editors of *THE JOURNAL* of the association, consisting of five members, one of whom shall be designated the chairman, shall be appointed by the president with the advice and consent of the Executive Committee. These members shall serve for a period of five years, a member being appointed each year.

12. No fertilizer definition or interpretation shall be adopted as tentative nor shall a tentative definition or interpretation be amended until such definition or interpretation has been recommended for adoption or amendment by the Committee on Definitions of Terms and Interpretation of Results on Fertilizers and published in the proceedings of the association.

13. No fertilizer definition or interpretation shall be adopted as official nor shall an official definition or interpretation be amended until such definition or interpretation has been recommended for adoption or amendment by the Committee on Definitions of Terms and Interpretation of Results on Fertilizers at two annual meetings.

REPORT OF AUDITING COMMITTEE.

The Auditing Committee has examined the accounts of *The Journal* and *Methods of Analysis*, covering the period from October 1, 1926, to October 1, 1927, and found the same to be correct as reported.

The committee has also examined the accounts of W. W. Skinner, Secretary-Treasurer, covering the period from October 1, 1926, to October 15, 1927, and found the same to be correct as reported.

J. W. SAMPLE.

J. J. SKINNER.

J. B. WEEMS.

Approved.

REPORT OF NOMINATING COMMITTEE.

The Nominating Committee desires to place in nomination the following names:

President: OSWALD SCHREINER, Bureau of Chemistry and Soils, Washington, D. C.

Vice-President: H. B. McDONNELL, Agricultural Experiment Station, College Park, Md.

Secretary-Treasurer: W. W. SKINNER, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee:

E. M. BAILEY, Agricultural Experiment Station, New Haven, Conn.

L. D. HAIGH, Agricultural Experiment Station, Columbia, Mo.

F. C. BLANCK, Bureau of Chemistry and Soils, Washington, D. C.

Ex-Officio Member of Executive Committee:

W. H. MACINTIRE, Agricultural Experiment Station, Knoxville, Tenn.

H. H. HANSON.

R. N. BRACKETT.

C. H. JONES.

It was moved, seconded, and carried that the secretary be directed to cast a unanimous ballot for the officers nominated.

W. H. MacIntire: I assure you that I am most happy to extend the gavel to my successor, Dr. Schreiner. Dr. Ross, will you and Dr. Brackett escort Dr. Schreiner to the platform? Members of the association, I present to you your duly elected president.

O. Schreiner: I thank you from the fullness of my heart for the honor that you are bestowing upon me. We have no time to hear a speech, but I assure you that I shall be most happy to serve the association in this capacity. Thank you.

Following remarks relating to the presentation of reports and papers by W. F. Hand, it was moved, seconded, and carried that members and others who address the association be requested to present their papers in abstract form and make use of lantern slides.

W. H. MacIntire: Before hearing the report of the Committee on Resolutions, I wish to state that before the meeting I recommended to the Executive Committee that a Committee on Necrology be appointed. The thought was that it was obviously unfair to leave this work for the Committee on Resolutions to do during the meeting, when time is so limited and often it is not possible to collect the necessary information

to make a complete report. The Executive Committee approved, and the following committee was appointed: W. W. Randall, C. A. Browne, and H. C. Lythgoe. The personnel of the Committee on Resolutions is the same. Dr. Randall will report for both committees at this time.

REPORT OF COMMITTEE ON NECROLOGY.

It is the painful duty of the committee in this, its first report, to state that the four following members of the association have died since the last annual meeting:

Frederick Belding Power, at Washington, March 26, 1927.

Julius Hortvet, at Minneapolis, April 7, 1927.

Harry Snyder, at Minneapolis, October 11, 1927.

E. H. Golaz, at Austin, Texas, October 14, 1927.

FREDERICK BELDING POWER.

This association has suffered a serious loss in the recent death of one of its most distinguished members: Frederick B. Power, noted worker in the fields of plant chemistry and pharmacy. He was born at Hudson, New York, in 1853, and, after several years' practical experience as an apothecary, was graduated from the Philadelphia College of Pharmacy in 1874. His chemical and pharmaceutical education was completed at the University of Strassburg, from which he obtained his doctor's degree in 1880. After returning to the United States, he established, in 1883, the School of Pharmacy at the University of Wisconsin, where he remained as dean until his resignation in 1892. He was scientific director of the technical laboratories of Fritsche Brothers from 1892 to 1896, and director of the Wellcome Research Laboratories of London from 1896 to 1914. One of Power's outstanding accomplishments was the conspicuous part which he played in the successive revisions of both the American and the British Pharmacopeia.

During the last ten years of his life Power was chief of the Phytochemical Laboratory of the Bureau of Chemistry and in this period was a frequent attendant at the meetings of this association—which profited greatly by his advice in devising methods for the analysis of rarer plant constituents.

Power was the author of some 150 scientific papers which secured for him world-wide recognition. He was a member of the National Academy of Sciences and the recipient of many degrees and other honors. He possessed the qualities of patience, thoroughness, accuracy, and love for his work, which should be the natural endowment of every scientist. He was the severest critic of his own work, and few chemists apply as many

checks as did he in the confirmation of his results. His friends, both in this association and elsewhere, will long remember his kindly, modest disposition, his simplicity of manner, and the spirit of helpfulness that he extended to every one in his wide acquaintance.

JULIUS HORTVET.

With his appointment, in 1912, as Referee on Food Adulterations and Associate Referee on Dairy Products, Julius Hortvet began the preparation of a series of reports which, year after year, bore the marks of an extraordinary diligence and acumen. He called freely upon others to assist him in collaborative work; his own part was always laborious, and his personal contributions—whether in novel suggestions, in painstaking research, or in the matter of reconciling divergent views—were usually essential. He realized the importance of the field over which he had been made overseer and strove with enthusiasm, and yet with patience and caution, to establish the work of the association upon a secure foundation.

As the senior member representing the association upon the Joint Committee on Definitions and Standards, Hortvet showed many of the same qualities. Here he was most judicial—carefully and impartially weighing the evidence, and often refusing to come to a decision because of what he regarded as an insufficient volume of essential data. His knowledge was exceptionally wide and accurate, and this was in itself sufficient to prevent his being swept away from safe moorings by assertions which he recognized as rash and unconvincing.

To all who came in contact with him, Hortvet exhibited great charm. Courteous, kindly, with a playful humor, yet sincere and thoroughly honest, he made no enemies and won a host of friends. His was a place that will not soon be filled¹.

HARRY SNYDER.

Harry Snyder, through many years of a busy life, was regarded as probably the foremost American authority in the field of the industrial chemistry of cereals. Born in New York State sixty years ago and a graduate of Cornell University (1889), his career had been run chiefly in the milling center, Minneapolis. For thirty-six years he was busily studying the chemistry of cereal products, developing new and more accurate methods for their analysis and tabulating and correlating analytical data of proved exactness. He was the author of several books dealing with soils, fertilizers, vegetable and animal foods, dairy products, nutrition; in his special field he wrote voluminously for scientific and trade journals. He was frequently present at the meetings of this asso-

¹ A sketch of Julius Hortvet has been published. See *This Journal*, 1927, 10: No. 3, iii.

ciation, and he was chosen its president in 1908. He contributed much to the value of discussions where his expert knowledge could be brought into play. During a period of years he reported, as referee, on soil and ash analysis, on vegetable proteids, etc.

He was a man of courteous manner and of most amiable disposition.

This committee hopes that there may soon be published in the *Journal* an account of the life and work of Harry Snyder, which will do justice to his claims upon the admiration of his associates.

E. H. GOLAZ.

E. H. Golaz, a Swiss by birth, received his scientific training in his native country and in France, coming to America when about 25 years of age. From 1905 to 1911, he was Professor of Materia Medica and Therapeutics at the Southern Methodist University, at Dallas, Texas. He was appointed Chief Chemist of the Food and Drug Department of the State of Texas in 1911, became Food and Drug Commissioner of the State in 1916, and served in that capacity until his death.

Golaz was one of those scientists whose knowledge was so profound and whose experience was so broad that modesty was his marked characteristic.

W. W. RANDALL.

C. A. BROWNE.

H. C. LYTHGOE.

Approved.

REPORT OF COMMITTEE ON RESOLUTIONS.

(1) *Resolved:* That the Association of Official Agricultural Chemists extends its felicitations to its honorary president and voices its hope that he may continue in excellent health and spirits.

(2) *Resolved:* That the association desires to express its grateful appreciation of the skill and courtesy shown by the president during this meeting, and its hope that his health may soon be restored.

(3) *Resolved:* That the association desires to express its appreciation of the painstaking work of its secretary, W. W. Skinner, and of its other officers and their assistants in making preparations for this annual meeting.

(4) *Resolved:* That to R. W. Balcom and his assistant editors, and to Miss Marian E. Lapp, the association owes a debt of gratitude for the efficient way in which they have conducted the affairs of *The Journal*.

(5) *Resolved:* That this association extends its thanks to R. W. Dunlap, the Assistant Secretary of Agriculture, for his courtesy in attending its convention and greeting its members.

(6) *Resolved:* That to the management of the Raleigh Hotel the thanks of this association are due, in recognition of the many courtesies extended during this annual meeting.

W. W. RANDALL.

C. A. BROWNE.

H. C. LYTHGOE.

Approved.

The meeting adjourned at 4.25 p. m., when all the reports had been presented. The proceedings for Monday and Tuesday will be published in Nos. 2, 3, and 4 of Volume XI.

CONTRIBUTED PAPERS.

OBSERVATIONS ON THE GUTZEIT METHOD FOR THE DETERMINATION OF ARSENIC¹.

By H. HEIDENHAIN (Wenatchee, Wash.).

During a year's experience with the Gutzeit method for the determination of arsenic on sprayed fruit, a number of observations that led to investigations as to the reliability of this method were made. The results are believed to be important.

The basis of the method used in this work was a modified form of the Gutzeit method that was formulated by the Baltimore Station of the U. S. Bureau of Chemistry. The essential features of this method have since been published². The writer, however, made one radical change by substituting the direction, "Compare the stain with similar stains produced under like conditions with known quantities of arsenic", for the direction, "Run known arsenic control with each batch".

The former clause directs the analyst to provide for the "like conditions" in his operations, but the latter clause permits no such liberty. It directs him to run controls with known quantities of arsenic with each batch. In so doing, the "like conditions" are automatically provided; in other words, new standards are prepared for each single test or set of tests to be run simultaneously.

While this is the ideal way, the method has been made cumbersome by this provision. Besides, it will often happen that the quantity of arsenic found is not within the range of the control tests, and the whole procedure must be repeated. The expenditure of time, work, and material is so great, if those directions are followed, that the method is not suitable for commercial purposes.

The analyst, naturally, will try to simplify his work, thinking that by careful observation of all details of the method—the volumes, the weights, and temperature—it should be possible to prepare standards and run tests under practically "like conditions" and thereby avoid the preparation of new standards with each batch of tests.

As a matter of fact, the writer found that duplicate standards made at intervals of a few days were for all intents and purposes identical and that such standards could be used for short periods as a basis for the determination of the unknown quantity.

¹ Presented at the 43d Annual Convention of the Association of Official Agricultural Chemists, held in Washington, D. C., November 1, 1927, by W. F. Clarke.

² *This Journal*, 1927, 10: 425.

INFLUENCE OF TEMPERATURE.

However, as time progressed, the gradual increase of the length of the stains observed was hard to explain. The first thought was that the sensitized paper had deteriorated, but this was found not to be the case; on the contrary, the paper was found to be very permanent. The probable cause for the increased length of the stains became apparent, however, when the tests and new standards were made after a decided drop in temperature. The lower temperature prevailing during the test seemed to affect the coloration of the paper strips. The temperature of the solution in the generator was lower at the end of the test than at the beginning. This, however, did not explain the phenomenon, because this condition would tend to shorten instead of lengthen the stain, as was shown by running parallel tests with different initial temperatures of the solutions. At the lower temperature the gas is evolved more slowly, and therefore more time is given for the hydrogen arsenide to react on the sensitized paper. Complete absorption takes place in the lower part of the strip, while a more rapid evolution of gas spreads the zone of absorption and makes the color longer and fainter.

Another possibility—that the absorption apparatus did not function alike at different temperatures—remains. The gas passes through moist cotton, and it may be assumed that it leaves the cotton fully saturated with moisture. Therefore, the lower the temperature of the moist cotton, the less the moisture of the gas will be. While but little moisture is required for the reaction of hydrogen arsenide on mercuric bromide—in fact the reaction takes place only in a concentrated solution of this reagent—it may be taken for granted that in the absence of all moisture no reaction would take place. Obviously a low temperature does not provide sufficient moisture for a vigorous reaction, and hence the faint, drawn-out colors at low temperatures.

To prove the correctness of this contention, the writer made a contrivance by which the temperature of the absorption apparatus could be controlled while the generator was left at the temperature of the room. When the temperature of the absorption apparatus was kept at 25°C., colors identical in length and intensity with those obtained during the warmer season were obtained.

These experiments show that it is not sufficient to start the generation of the gas at a certain temperature of the solution, but that the temperature of the absorption apparatus must likewise be maintained and controlled if comparable results are to be obtained. They also show that temperature affects the function of these two parts of the apparatus in the opposite direction. Lower temperatures of the solution in the generator produce shorter and more intense colors than higher temperatures, while lower temperatures in the absorption apparatus produce faint, long-drawn-out colors with less defined end points.

To make use of these observations in a practical way would require an arrangement that would control the temperature of the generator as well as that of the absorption tubes. This can be done but not very conveniently. It occurred to the writer that since the temperature of a water bath is more easily controlled than that of an air bath, submersion of the whole apparatus in water would be the easiest way out of the difficulty. The process was tried at the temperatures of 15°, 20°, 25°, and 30°C. The apparatus was submerged so that only 3 cm. of the tube carrying the sensitized paper extended above the water. The results showed that the length of the colors was nearly the same at these widely varying temperatures. Evidently the two opposite effects had nearly balanced each other. But the higher the temperature the stronger was the color and the better defined was the end point.

To simplify the method it should be so modified that the establishment of permanent standards is permissible, and this can be done if the temperature of all parts of the apparatus is controlled. A high temperature as a standard, say 30°C., would be preferable to a medium or low temperature, because the colors then are more satisfying. It was found that a 5 gallon bath keeps very permanent during the time required for the reactions with no more attention than having the temperature right at the start. On account of the balancing effect of the temperature a slight variation is immaterial.

SULFURIC VERSUS HYDROCHLORIC ACID.

The official method¹ gives the option of sulfuric or hydrochloric acid. It then proceeds: "Heat to 90°C., add 3 drops of the stannous chloride solution, and heat for 10 minutes. Cool, etc".

The modification issued by the Bureau of Chemistry a year ago, to which reference was made in the second paragraph of this paper, reads as follows: "Add 5 cc. of concentrated sulfuric acid in total volume of 40 cc. Heat rapidly in water bath until bath reaches 90°C. Allow to stand not more than 5 minutes. Cool rapidly until sample reaches 18°C. Discard determinations where solution becomes cloudy during reduction or where lead acetate paper becomes gray differing from normal black sulfide stain or where evolution of gas is not reasonably vigorous".

It will be noticed that the modification does not mention heating the solution after the addition of the stannous chloride. However, as these directions were intended to give only changes in the official method, it could be understood that in this respect the original official version should be followed.

Not understanding it that way, the writer had used the method without heating after the addition of the stannous chloride. Standards

¹ *Methods of Analysis*, A. O. A. C., 1925, 173.

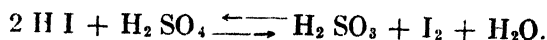
were prepared with both acids, and they were found to be identical and satisfactory. Therefore, no attention was given to this point until it was learned that the results obtained were higher than those obtained by other chemists. In searching for the cause, comparative tests were made with and without heating for 10 minutes after addition of the stannous chloride, and it was found that such heating is not permissible when sulfuric acid is used. The results are far too low, as shown by the following figures:

As USED	As FOUND	
	WITHOUT	WITH
	Heating after addition of Stannous Chloride	
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
8	8	4
16	16.5	8
24	24	9
32	30.5	11.5
40	43	10
48	39.5	11.5

A number of solutions obtained from the oxidation of 40 grams of potato pulp in the presence of known quantities of arsenic being available, the writer repeated the experiment with the following results:

DIGESTION OF PULP IN PRESENCE OF As:	QUANTITY (1/50) USED FOR TEST CONTAINING—	ARSENIC (As) FOUND	
		WITHOUT	WITH
		Heating after addition of Stannous Chloride	
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
250	5	7.5	2
500	10	10	5
1000	20	20	7
1500	30	29	7
2000	40	40	9.5
2500	50	47	9

When the generators were disconnected at the end of the process an odor of hydrogen sulfide was emanating from the solutions that had been heated an additional 10 minutes. As the other solutions had no such odor, it was decided that the hydrogen sulfide caused the low results. It is possible that part of the arsenic is converted to arsenic trisulfide, which is not subject to reduction by the zinc. That more hydrogen sulfide is formed when heating is continued in the presence of stannous chloride is due to a reaction of the process, which is as follows:



This reaction goes on to a state of equilibrium. When stannous chloride is added the free iodine is removed, and the reaction goes on, producing more sulfurous acid. This is reduced by the zinc to hydrogen sulfide. A small quantity of sulfurous acid does not affect the method, but the larger quantity produced by longer heating in the presence of stannous chloride renders the results worthless.

When hydrochloric acid is used this process is not liable to go on except when large aliquots of the arsenic solution are used.

The opinion of the writer is that the 10 minute heating after addition of stannous chloride answers no good purpose and should be abandoned, no matter whether sulfuric or hydrochloric acid is used for the evolution of the gas.

PREPARATION OF SOLUTION.

It is uncertain whether or not it is necessary to carry the oxidation of the organic matter to completion, but as there is no difficulty in obtaining a colorless solution it seems superfluous to investigate this point. One particular observation in this part of the process was that it is not sufficient to heat the acid solution until heavy white fumes evolve. When this stage was reached it was found to be necessary to raise the heat so that the sulfuric acid is kept boiling for 10 minutes. Too early interruption of the heating of the acid solution tends to lower the results, which is especially noticeable when large aliquots of the solution are used. The acid was boiled before and after dilution with water.

SUGGESTIONS FOR A MODIFIED METHOD.

In conclusion, the outlines of the method as the writer thinks it ought to be formulated so that it is safe in the hands of any analyst are given.

REAGENTS.

No change.

APPARATUS.

Substitute a 100 cc. Erlenmeyer flask for the salt mouth bottle shown in Fig. 11.

PREPARATION OF SOLUTION.

Heat the peelings of the fruit in a 500 cc. Erlenmeyer flask of Pyrex glass with 15 to 20 cc. of concentrated sulfuric acid and 35 cc. of concentrated nitric acid. Should the bulk of the peelings be too great, divide between two flasks, charging each flask with the quantities of the acids given previously. Heat at first moderately. When strong action is over, raise the heat. When the solution turns brown, add nitric acid in small portions until the solution remains slightly yellow, but free from any brownish tint. Toward the end of the oxidizing process add at one time from a dropping bottle a few drops of concentrated nitric acid. Avoid charring or allowing the mass to turn black. When oxidation is finished, raise the heat until the acid boils and boil gently for 10 minutes. Cool. Add 100 cc. of water, boil down until heavy white fumes evolve, raise heat to boiling, and boil gently for 10 minutes. Cool. Dilute to a convenient volume.

DETERMINATION.

Introduce an aliquot of the solution containing from 10 to 50 mmg. of arsenic, or from 15 to 70 mmg. of arsenic trioxide into the generator flask. Add 10 cc. of concentrated sulfuric acid or 10 cc. of strong hydrochloric acid. Add water to bring the volume of solution up to 75 cc. Add 4 cc. of 20 per cent potassium iodide solution. Heat in a boiling water bath to 90°C. Remove from bath. Add three drops of stannous chloride solution. Cool rapidly to 30°C. Add 15 grams of stick zinc or coarse zinc shot (not granulated zinc), which has been previously used for this purpose. Connect the absorption tubes. Slip a heavy metal ring over the flask. Submerge the whole apparatus within 3 cm. from the end of the tubes containing the sensitized paper in water of 30°C. and hold therein for 1 hour and 30 minutes.

Remove the paper strips and compare the stain with stains produced under like conditions with known quantities of arsenic. Use the extreme yellows of the stain as the end point, read both sides of the stains, and compute the average of the two readings.

DETECTION AND DETERMINATION OF ADDED
MOISTURE IN SAUSAGE.

By PERCY A. SIGLER (Meat Inspection Division, Bureau of Animal Industry, Washington, D. C.).

The meat content of sausage is largely of the less expensive portions removed in preparing carcasses and cuts for the trade. Wholesale cuts—chucks, plates, and flanks in the case of beef, and shoulder butts in the case of pork—and meat by-products, which include a variety of organs and parts not included in the term “meat”¹, are utilized. Sometimes entire carcasses are used.

The usual adulterants of sausage are water and cereal. Because the meat and meat by-products used are finely ground or chopped, as a rule, it is possible to add liberal quantities of water without ready detection by the consumer. Ground lean meat, especially, can be made to take up a considerable quantity. When cereal flour is used as a constituent, a still larger quantity of water may be incorporated in the product. Adulterated products made in imitation of sausage have been known to contain up to 15 per cent or more of cereal and as much as 40 per cent of added water. Any adequate inspection of sausage, therefore, must include the detection and determination of added water.

To detect the presence and determine the quantity of added moisture in sausage it is necessary for the analyst to know the normal moisture content of the various meats and meat by-products used in its preparation. Many analyses of the various cuts and internal organs of the different meat animals have been published, but little if any mention is made in the literature of analyses of the various meat by-products used in the manufacture of sausage. These analyses show that there is

¹ U S Dept Agr Food Inspection Decision 205, 1926.

a wide variation in the moisture content of the same wholesale cuts from different animals of the same species, as well as from different cuts from the same animals, and that the variation is due largely to a difference in fat content, the moisture content varying inversely with the percentage of fat present. For example, the wholesale cuts from a fat steer invariably contain less moisture than the same cuts from a lean animal. This relation, which holds for other animals but is especially true in the case of hogs, has long been known by chemists who have investigated the composition of the meats of domestic animals.

König¹ states that as the fat content of meat increases, the percentages of moisture and other constituents decrease. Analyses of different cuts from the same animal show that the cut containing the most fat contains the least moisture and vice versa. It has also been noted by different investigators that when the meat is freed from intermuscular fat, there is practically no difference in the water content of meat from the same class of animals and but little difference in the moisture content of fat-free meat from different species of animals. Some of these results given by König are shown in Table 1.

TABLE 1.
Moisture content of fat-free meat.

FLESH FROM—	WATER, FAT-FREE BASIS	ANALYST
	<i>per cent</i>	
Cattle	76.59	Petersen
Cattle	76.21	Nowack
Cattle	75.86	Voit
Cattle	75.40	Ruppert
Calf	78.85	Petersen
Sheep	76.67	Petersen
Hog	74.24	Petersen
Horse	74.76	Petersen
Horse	74.04	Nowack
Rabbit	74.00	Meyer

Results of similar observations have recently been summarized by Chatfield².

Prompted by the lack of published data concerning the composition of the commonly used sausage materials, E. A. Boyer, Chemist in Charge, Omaha Meat Inspection Laboratory, Bureau of Animal Industry, conducted a series of analyses of these materials and showed the consistency of the moisture content on a fat-free basis. The results given in Table 2 are taken from an unpublished report made by Boyer in 1912 to the Bureau of Animal Industry.

¹ *Chemie der Nahrungs- und Genussmittel sowie der Gebrauchsgegenstände*, Vol. 2, 5th ed., 1920.

² U. S. Dept. Agr. Cir. 380, 1926.

TABLE 2.
Composition of sausage materials.
(A)—FROM CATTLE.

MATERIAL	WATER	FAT	PROTEIN	WATER, FAT-FREE BASIS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1—FRESH				
Lean Plate Trimmings.....	71.98	7.88	18.24	78.14
Lean Plate Trimmings.....	76.66	2.06	19.84	78.19
Chuck Trimmings.....	75.15	4.33	19.86	78.56
Chuck Trimmings.....	76.67	1.79	20.46	78.07
Chuck Trimmings.....	70.93	8.72	19.68	77.70
Hind Shank Meat.....	71.68	6.14	20.63	76.37
Hind Shank Meat.....	69.37	8.93	20.78	76.18
Front Shank Meat.....	72.14	5.66	20.89	77.63
Front Shank Meat.....	72.27	6.21	20.91	77.05
Brisket Meat.....	64.86	16.46	17.10	77.64
Brisket Meat.....	68.80	12.11	17.91	78.28
Tongue Trimmings.....	71.47	9.78	18.07	79.22
Tongue Trimmings.....	68.06	13.31	18.34	78.51
Tongue Glands.....	61.55	20.39	15.63	77.31
Cheek Meat.....	71.76	7.85	18.15	77.87
Cheek Meat.....	73.23	8.05	18.53	79.64
Head Meat.....	67.34	13.28	17.75	77.65
Head Meat.....	72.02	8.80	18.03	78.97
Ox Lips.....	68.21	11.98	17.16	77.49
Ox Lips.....	68.07	14.47	16.69	79.58
Weasand Meat.....	75.81	5.85	14.35	80.52
Weasand Meat.....	78.33	5.80	13.69	83.15
Weasand Meat.....	75.74	6.85	15.66	81.31
Hearts.....	74.71	6.55	17.37	79.94
Hearts.....	75.05	6.53	16.50	80.29
Heart Valves.....	77.53	5.13	15.38	81.74
Heart Valves.....	74.07	10.80	13.25	83.04
Cooked Tripe.....	80.22	2.38	17.25	82.18
Cooked Tripe.....	80.20	2.32	16.97	82.10
Liver.....	69.52	4.66	19.28	72.92
Liver.....	70.06	4.24	20.59	73.16
Hanging Tenderloins.....	64.06	16.20	17.22	76.43
Hanging Tenderloins.....	67.43	13.01	18.22	77.51
Small Trimmings.....	65.19	15.34	18.47	77.00
Small Trimmings.....	68.39	13.23	17.28	78.82
2—FROZEN				
Chucks..... (3 Months)	65.36	12.67	20.25	74.84
Cheek Meat..... (98 Days)	69.56	9.23	21.00	76.63
Blade Trimmings..... (138 Days)	59.14	21.04	17.81	74.89
Regular Trimmings..... (3 Months)	56.35	25.80	16.25	76.05
Regular Trimmings..... (6 Months)	63.87	15.41	18.94	75.51
Hind Shank Meat..... (4 Months)	71.68	6.39	20.44	76.57
Hearts..... (1 Month)	75.31	5.62	18.13	79.79
Hearts..... (3 Months)	73.21	6.99	17.56	78.71
Livers..... (1 Month)	67.40	5.90	22.06	71.63
3—CURED				
	<i>days</i>			
D. C. Small Trimmings*.....	75	62.79	12.86	72.06
D. C. Head Meat.....	18	67.24	7.47	72.66
D. C. Cheek Meat.....	7	65.10	11.34	73.43
D. C. Cheek Meat.....	57	68.06	5.93	72.35
D. C. Weasands.....	10	70.32	3.11	72.58

* Dry cured.

TABLE 2.—Continued.
Composition of sausage materials.
(A)—FROM CATTLE—Continued.

MATERIAL		WATER	FAT	PROTEIN	WATER, FAT-FREE BASIS
3—CURED—Continued.	days	per cent	per cent	per cent	per cent
P. C. Weasands**	6	73.69	6.05	78.44
D. C. Weasands*	6	73.09	4.78	76.77
D. C. Shank Meat	138	68.12	6.59	72.93
D. C. Chucks	6	64.08	9.70	70.96
D. C. Hearts	95	72.57	5.40	76.50
P. C. Hearts**	60	65.87	7.07	70.88
D. C. Regular Trimmings*	90	60.90	12.61	69.69
P. C. Ox Lips**	12	65.24	12.22	74.32
P. C. Heart Valves	8	70.98	10.31	79.14
Trimmings from Cured Beef Hams	22	74.26	3.99	..	77.35

(B)—FROM CALVES.

1 -FRESH				
Whole Hind Quarter.....	76.61	1.75	21.00	77.97
Whole Hind Quarter.....	77.04	1.69	20.35	78.36
Whole Front Quarter.....	75.50	3.37	20.00	78.13
Whole Front Quarter.....	75.70	3.44	20.75	78.39

(C)—FROM SWINE.

1 --FRESH				
Head Meat	71.86	6.88	19.84	77.1
Head Meat	71.35	7.19	19.19	76.8
Cheek Meat.	64.23	16.78	17.26	77.1
Cheek Meat.	68.23	13.38	17.53	78.7
Jowl Trimmings	39.50	49.38	9.51	78.0
Jowl Trimmings	42.92	44.35	10.75	77.1
Fat, Jowl Meat	24.14	68.95	6.44	77.7
Fat, Jowl Meat	26.29	66.43	6.47	78.3
Snouts.	58.16	26.17	14.75	78.7
Snouts.	58.57	25.71	14.97	78.8
Lean Shoulder Trimmings	63.28	18.65	16.63	77.7
Fat Shoulder Trimmings	46.05	41.05	11.25	78.1
Fat Shoulder Trimmings	38.96	50.39	10.03	78.5
Fat Shoulder Trimmings	47.65	40.39	11.19	79.9
Lean Back Bone Trimmings	56.09	26.18	15.66	75.9
Lean Back Bone Trimmings	56.37	27.71	15.69	77.9
Neck Bone Trimmings	46.09	39.99	12.86	76.8
Neck Bone Trimmings	54.85	30.25	13.59	78.6
Lean Neck Bone Trimmings	66.19	15.49	17.50	78.3
Lean Butts	64.07	17.32	17.38	77.4
Lean Butts	66.20	15.63	16.50	78.4
Medium Fat Belly Trimmings	43.54	42.86	11.59	76.1
Medium Fat Belly Trimmings	43.72	43.89	10.81	77.9
Lean Ham Trimmings	70.26	7.56	20.38	76.0
Lean Ham Trimmings	69.37	10.90	18.13	77.8
Weasand Meat	75.53	4.85	18.25	79.1
Tongues	63.31	20.86	14.22	78.0
Tongues	65.53	18.59	13.97	80.5
Livers	69.22	5.07	17.85	72.0
Livers	71.23	6.51	16.81	76.1
Hearts	69.96	13.78	14.90	81.1
Hearts	75.91	6.61	15.76	81.2
Weasands	79.10	5.32	15.09	83.5

* Dry cured.
** Pickle cured.

TABLE 2.—Continued.
Composition of sausage materials.
(C)—FROM SWINE—Continued.

MATERIAL		WATER	FAT	PROTEIN	WATER, FAT-FREE BASIS
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
2—FROZEN					
	<i>days</i>				
Butts	124	58.88	22.89	15.59	76.36
Snouts	120	58.25	24.45	16.81	77.10
Cheek Meat	93	66.30	14.83	17.63	77.84
Regular Trimmings	42	41.64	46.51	11.00	77.84
Lean Trimmings	37	45.76	40.82	12.94	77.32
Lean Trimmings	27	64.06	16.55	18.56	76.76
Hearts	44	74.38	6.88	16.94	79.87
Hearts	60	72.62	8.90	16.06	79.71
3—CURED					
P. C. Tongues**	21	58.81	17.77	...	71.52
P. C. Snouts	90	52.28	24.27	...	69.03
P. C. Snouts	12	49.88	27.41	...	68.71
D. C. Berliner Trimmings*					
Retrimmed	30	55.69	23.19	...	72.50
D. C. Hearts	58	71.89	5.39	...	75.98
P. C. Hearts**	8	61.40	9.17	...	67.60
D. C. Cheek Meat*	37	65.49	7.07	...	70.47
D. C. Cheek Meat	14	64.95	13.25	...	74.87
D. C. Lean Butts	53	58.00	19.36	...	71.92
P. C. Meat Trimmings (Mixed Ages)**		61.43	20.94	...	77.70
P. C. Meat Trimmings (Mixed Ages)		65.58	12.19	...	74.68
P. C. Meat Trimmings (Mixed Ages)		66.80	13.41	...	77.14
P. C. Pork Ears	4	56.51	18.26	...	69.13
D. C. Shoulder Trimmings*	18	66.51	9.89	...	73.82
P. C. Belly Trimmings (Mixed Ages)**		47.76	39.38	...	78.78
D. C. Lean Trimmings*	11	63.22	13.49	...	73.08
P. C. Weasands**	6	72.51	2.99	...	74.72

(D)—FROM SHEEP.

1—FRESH					
Cheek Meat		64.91	18.75	15.66	79.89
Cheek Meat		72.27	7.88	19.34	78.45
Hearts		74.29	8.23	15.84	80.49
Hearts		68.08	15.95	13.75	81.00
Tongues		63.50	23.08	12.04	82.55
Tongues		69.07	14.61	14.63	80.89
Lips		71.75	10.90	15.63	80.53
2—FROZEN					
	<i>days</i>				
Cheek Meat	39	66.13	15.47	17.19	78.23
Hearts	41	69.49	13.07	16.06	79.94
3—CURED					
P. C. Hearts**	9	63.70	15.10	75.03

* Dry cured.

** Pickle cured.

The considerable differences in the absolute moisture content of the different meats and meat by-products in sausage which make it impossible to base any standard for the water content of sausage on total

moisture will be seen in Table 2 to vary from 24 per cent in the case of fat pork jowls to 80 per cent in cooked beef tripe. In general, this difference is in proportion to the fat content, which calculated on a fat-free basis varies, with but few exceptions, between 77 per cent and 79 per cent. The principal exceptions are noted in cured meats, in which a variation is to be expected, owing to the loss of water by evaporation during smoking and dehydration by the salt.

The water content of meats and meat by-products on a fat-free basis being nearly constant, it would seem to follow directly that the relation of water to protein should be constant. The ratio of protein to moisture, or of non-fatty organic matter to moisture in meats, has also been studied and determined by a number of chemists. Feder¹ recommended the use of the ratio between the fat-free organic matter and water as a basis for this determination, pointing out that it is fairly constant for fresh meats and in the case of beef or pork does not vary appreciably from 1 : 4.0. Feder confirmed his work later as did other investigators, so that this ratio of non-fatty organic matter to moisture is now a generally accepted standard for normal meat.

Grossfeld² found that the protein content ($N \times 6.25$) of meat did not differ appreciably from that of the organic non-fatty material and so recommended the use of the same ratio for protein and moisture (1 : 4.0) as a standard for the detection of added water, remarking that the value for protein is more quickly, simply, and cheaply obtained than that for fat-free organic matter.

Pannwitz and Harder³ report the analyses of 102 samples of ground fresh beef, of which only 2 samples show protein-moisture ratios wider than 1 : 4.0, and it was found that these contained added water.

Hoagland and Powick⁴ found that the protein-moisture ratio of flesh from extremely emaciated cattle ranges from 1 : 3.8 to 1 : 4.7, the average being 1 : 4.2. Feder⁵ also found that the protein-moisture ratio is much higher in flesh of emaciated cattle than in normal flesh. However, this exception should not be considered a reason for tolerance. Emaciation being a pathological condition, the Federal Meat Inspection regulations⁶ justly require the condemnation of emaciated animals as unfit for human food.

The constancy of the protein-moisture ratio in sausage materials is clearly shown in Table 3. The analyses of the different sausage materials were made by E. A. Boyer, and they include, in addition to the complete analyses of the various portions, results calculated from the analyses showing the percentage of water in the fat-ash-free substance and the

¹ *Z. Nahr. Genussm.*, 1913, 25: 577.

² *Ibid.*, 1921, 42: 173.

³ *Ibid.*, 1922, 44: 344.

⁴ *J. Agr. Research*, 1925, 31: 1001.

⁵ *Z. Nahr. Genussm.*, 1922, 43: 193.

⁶ U. S. Bur. Animal Industry Order 211 (revised). Reg. 11, Sec. 19.

TABLE 3.
Protein-moisture ratio in sausage materials.
 (A)—FRESH CHILLED MEATS USED IN PORK SAUSAGE.

DESCRIPTION	WATER	FAT	PROTEIN (N x 6.25)	ASH	WATER IN FAT-ASH-FREE SUBSTANCE	RATIO OF PROTEIN TO WATER
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Neck Bone Trimmings	55.0	30.1	13.6	0.7	79.5	1 : 4.0
Neck Bone Trimmings	53.6	31.8	13.8	0.8	79.5	1 : 3.9
Neck Bone Trimmings	54.4	30.8	13.7	0.8	79.5	1 : 3.9
Back Fat Trimmings	48.3	38.4	12.8	0.6	79.2	1 : 3.8
Back Fat Trimmings	38.6	50.9	10.0	0.5	79.4	1 : 3.9
Back Fat Trimmings	45.0	43.0	11.3	0.6	79.8	1 : 4.0
Shoulder Trimmings	40.7	48.6	9.9	0.5	80.0	1 : 4.1
Shoulder Trimmings	42.6	45.6	11.5	0.6	79.2	1 : 3.7
Shoulder Trimmings	48.2	39.4	12.1	0.3	79.9	1 : 4.0
Belly Trimmings	52.4	33.1	13.6	0.7	79.2	1 : 3.9
Belly Trimmings	53.3	31.7	14.1	0.8	79.0	1 : 3.8
Belly Trimmings	57.9	26.0	14.7	1.0	79.3	1 : 3.9
Belly Trimmings	47.4	39.3	13.1	0.7	79.0	1 : 3.6
Jowl Trimmings	32.9	58.3	8.3	0.5	79.9	1 : 4.0
Jowl Trimmings	24.3	69.4	6.3	0.3	80.2	1 : 3.9
Jowl Trimmings	37.5	52.7	9.3	0.5	80.1	1 : 4.0
Ham Trimmings	48.1	37.2	13.6	0.7	77.4	1 : 3.5
Ham Trimmings	53.5	29.6	16.0	0.9	76.9	1 : 3.3
Ham Trimmings	54.2	28.6	16.1	1.0	77.0	1 : 3.4
Shoulder Fat	7.0	91.0	1.9*	0.1	78.7	1 : 3.7
Shoulder Fat	6.8	91.4	1.7*	0.1	80.0	1 : 4.0
Cheek Meat	71.7	7.3	19.5	1.0	78.2	1 : 3.7
Cheek Meat	73.9	4.8	19.6	1.0	78.4	1 : 3.8
Cheek Meat	71.0	8.0	19.9	0.9	77.9	1 : 3.6
Head Meat	63.5	17.9	17.7	0.8	78.1	1 : 3.6
Head Meat	63.1	19.6	16.4	0.9	79.4	1 : 3.8
Head Meat	69.3	10.2	19.3	0.9	78.0	1 : 3.6
Head Meat	54.5	29.0	15.3	0.8	77.6	1 : 3.6
Average					79.0	1 : 3.8

(B)—MATERIALS USED IN FRESH SAUSAGE OTHER THAN PORK SAUSAGE.

1—HOG CARCASS					
Regular Trimmings (21 Samples)	42.9	45.1	11.0	0.7	1 : 3.9—Average 1 : 4.0—Maximum 1 : 3.3—Minimum
Head Meat..... (4 Samples)	62.6	19.2	17.2	0.9	1 : 3.7—Average 1 : 3.8—Maximum 1 : 3.6—Minimum
Cheek Meat..... (4 Samples)	72.2	6.7	19.7	1.0	1 : 3.7—Average 1 : 3.8—Maximum 1 : 3.6—Minimum
Tongue Trimmings	68.6	13.2	17.0	0.9	1 : 4.0
Tongue Trimmings	70.1	12.0	16.6	0.8	1 : 4.2
Snouts.....	51.7	33.9	13.7	0.5	1 : 3.8
Snouts.....	51.6	34.8	12.9	0.5	1 : 4.0
Snouts.....	56.8	27.8	14.5	0.4	1 : 3.9
Ears.....	58.3	23.7	17.2	0.4	1 : 3.4
Ears.....	56.8	26.3	16.4	0.4	1 : 3.5
Ears.....	57.6	25.5	16.8	0.4	1 : 3.4

* Protein determined by difference.

TABLE 3.—Continued.

Protein-moisture ratio in sausage materials.

(B)—MATERIALS USED IN FRESH SAUSAGE OTHER THAN PORK SAUSAGE—Continued.

DESCRIPTION	WATER	FAT	PROTEIN (N \times 6.25)	ASH	RATIO OF PROTEIN TO WATER	REMARKS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
2—BEEF CARCASS						
Flanks.....	54.2	29.1	16.0	0.7	1 : 3.4	
Flanks.....	67.0	12.8	19.3	1.0	1 : 3.5	
Flanks.....	59.2	23.7	15.4	0.8	1 : 3.8	
Flanks.....	74.1	4.8	20.2	1.0	1 : 3.7	
Loins.....	67.8	12.9	18.1	0.9	1 : 3.7	
Loins.....	74.1	4.0	20.6	1.1	1 : 3.6	
Plates.....	79.6	0.7	19.1	0.9	1 : 4.2	
Plates.....	61.7	18.2	19.4	0.9	1 : 3.1	
Chucks.....	77.3	1.0	20.6	1.1	1 : 3.8	
Chucks.....	70.2	9.1	19.8	1.1	1 : 3.5	
Chucks.....	75.7	4.5	18.2	1.1	1 : 4.2	
Chucks.....	72.7	4.1	23.0	1.0	1 : 3.1	
Shanks.....	73.3	3.9	21.2	1.0	1 : 3.5	
Shanks.....	75.4	4.4	18.9	0.9	1 : 4.0	
Cheeks.....	74.5	3.9	19.8	0.9	1 : 3.8	
Cheeks.....	74.4	5.5	19.6	0.9	1 : 3.8	
Head Meat.....	73.1	7.9	18.6	0.9	1 : 3.9	
Head Meat.....	72.0	9.1	17.8	0.9	1 : 4.0	
Head Meat.....	74.1	6.0	19.0	1.0	1 : 3.8	
Tongue Trimmings	67.8	15.1	16.3	0.8	1 : 4.2	
Tongue Trimmings	77.0	2.5	19.4	0.9	1 : 4.0	
Tongue Trimmings	70.5	12.2	16.4	1.0	1 : 4.2	
Tongue Trimmings	71.3	10.0	17.8	0.8	1 : 4.0	

(C)—MEAT BY-PRODUCTS USED IN SAUSAGE.

1—HOG CARCASS						
Stomachs.....	79.6	4.8	15.0	0.8	1 : 5.3	Cleaned and chilled
Stomachs.....	77.8	6.2	14.7	0.5	1 : 5.3	Scalded and chilled
Stomachs.....	79.3	5.1	14.9	0.4	1 : 5.4	Ready for sausage
Spleens.....	76.1	5.1	16.8	1.6	1 : 4.5	Direct from carcass
Spleens.....	76.3	5.1	16.8	1.4	1 : 4.5	Direct from carcass
Hearts.....	75.4	6.5	16.3	1.0	1 : 4.6	From killing floor
Hearts.....	77.9	3.7	17.2	1.3	1 : 4.5	From killing floor
Hearts.....	77.3	4.4	17.0	1.3	1 : 4.5	From killing floor
2—BEEF CARCASS						
Hearts.....	78.9	3.2	15.9	1.1	1 : 5.0	Chilled one day
Hearts.....	79.4	3.0	15.9	1.1	1 : 5.0	Chilled one day
Hearts.....	80.0	2.0	16.1	1.1	1 : 5.0	Chilled one day
Weasands.....	72.5	6.9	19.3	1.1	1 : 3.7	Direct from carcass
Weasands.....	74.6	5.7	18.2	1.1	1 : 4.1	Direct from carcass
Weasands.....	78.6	5.5	14.9	0.7	1 : 5.3	Washed and chilled
Weasands.....	77.9	5.7	15.5	0.7	1 : 5.0	Washed and chilled
Spleens.....	78.6	1.9	17.7	1.5	1 : 4.4	From killing floor
Spleens.....	76.8	3.4	18.0	1.4	1 : 4.3	From killing floor
Aorta.....	73.2	3.6	22.4	0.6	1 : 3.3	From killing floor
Aorta.....	74.6	4.7	19.3	0.9	1 : 3.9	
Palates.....	70.8	11.7	16.4	0.9	1 : 4.3	
Lips.....	64.9	17.4	17.2	0.8	1 : 3.8	Fresh chilled
Lips.....	73.9	4.8	20.3	1.0	1 : 3.5	Fresh chilled
Lips.....	71.4	9.8	18.0	0.7	1 : 4.0	Fresh chilled

relation of protein to water. These results, heretofore unpublished, were reported to the Bureau in 1912.

From Table 3-(C) it will be noted that the protein-moisture ratio of the meat by-products in sausage is much wider than in meats. However, with the possible exception of weasands, aortas, palates, and lips, it is impossible to secure the samples in such a manner that analyses can be regarded as representing the composition of the parts in their natural condition. For instance, hearts and spleens will retain a considerable quantity of blood and hog stomachs will retain a quantity of the water used in their cleaning. Therefore it is not deemed advisable or necessary to make allowance for these parts.

As shown by the data recorded in Table 3-(A) the meats used in the manufacture of pork sausage have a water content approaching 7 per cent as a minimum and 74 per cent as a maximum. Therefore, a mixture of these meats in the varying proportions used in pork sausage, and consequently the finished products, will contain widely varying quantities of water.

A method of limited value has been proposed by Boyer for comparing the water content of sausage with the water content of the meats and meat by-products used therein. It is based on the observation that when the fat is eliminated, by calculating the results on a fat-free or fat-ash-free basis, the percentage of water in meats from all parts of carcasses shows only slight variation. This point is confirmed, in so far as the meats used in pork sausage are concerned, by the data recorded in Table 3-(A). According to the twenty-eight analyses recorded, calculated on a fat-ash-free basis, these meats contain approximately 79 per cent of water—minimum 77.0 per cent, maximum 80.2 per cent, and exceeding 80 per cent in only two instances.

The relation of protein to water shown in the data given in Table 3-(A) is of special interest because it suggests a means of comparing the water content of these meats with the sausage manufactured therefrom. The extremes of the ratio in the case of meats used in the manufacture of pork sausage, for example, are 1:3.3 and 1:4.1 and in general approach the ratio 1:4.0. In twenty-four of the twenty-eight analyses recorded the percentage of water is not less than 3.6 times and not greater than 4 times the percentage of protein.

When it is considered that the sausage meats listed in Table 3-(A) are used as components of a mixture, not separately, it is to be expected that the mixtures would show much less variation in the analyses, including the percentage of water in the fat-ash-free substance and the ratio of protein to water, than is shown by the individual portions.

Analyses of ten meat mixtures, made from samples of meats of the same history as those recorded in Table 3-(A), and mixed under laboratory conditions in the proportions found to be used at inspected estab-

lishments in the manufacture of pork sausage, as well as of ten meat mixtures, representing ten brands of pork sausage, commercially prepared for use at four meat packing establishments, were made by Boyer. The formulas of the ten meat mixtures prepared in the laboratory and results of all analyses are given in Tables 4 and 5.

TABLE 4.

Formulas of meat mixtures as prepared in laboratory.

- 1—Shoulder, jowl and back-fat trimmings in equal portions.
- 2—60 per cent back-fat trimmings, 40 per cent shoulder trimmings.
- 3—50 per cent shoulder trimmings, 30 per cent neck-bone trimmings, and 20 per cent ham trimmings.
- 4—Shoulder, jowl, and belly trimmings in equal portions.
- 5—50 per cent back-fat trimmings, 25 per cent lean neck-bone trimmings, and 25 per cent shoulder trimmings.
- 6—Lean shoulder trimmings and ham trimmings in equal portions.
- 7—Equal portions of ham, back-fat, and belly trimmings with 40 per cent jowl trimmings.
- 8—55 per cent shoulder trimmings, 45 per cent jowl trimmings.
- 9—Equal portions of neck-bone and shoulder trimmings with 12 per cent jowl trimmings.
- 10—Equal portions of ham, belly, jowl, shoulder, and back-fat trimmings.

The data recorded in Tables 4 and 5 offer confirmatory evidence of the slight variation in the water content of the meats or meat mixtures used in pork sausage when expressed on a fat-ash-free basis and also show that the ratio of protein to water is even less variable than noted for the individual portions, the extremes of the ratio being 1 : 3.6 and 1 : 4.1. Table 5-(B) not only assists in making practical application of preceding observations, but it also shows that there is no loss of the normal water of the meats previous to their use in pork sausage. These samples were taken immediately before the addition of the salt and spices.

As the data recorded in Tables 3 and 5 indicate that the relation of protein to water approaches the ratio of 1 : 4, it is evident that in average sausage any percentage of water in excess of four times the percentage of protein could be regarded as added water. The expression "added water" as used in this paper signifies water in excess of the quantity present in normal fresh or chilled meats and meat by-products. Further, the degree of excess would be an accurate indication of the percentage of added water in the sausage.

In applying these principles the details of three experiments out of a number showing concordant results, reported by Boyer, are given in Table 6. The meat mixtures used represent the average meat mixtures of pork sausage with a water content of approximately 79.5 per cent of the fat-ash-free substance and a ratio of protein to water of 1 : 3.9. To 100 grams of Meat Mixture A, 5 grams of water and 2 grams of seasoning were added to make Sausage A; to 100 grams of Meat Mixture B,

TABLE 5.
Analyses of pork sausage meat mixtures.
(A)—AS PREPARED IN LABORATORY.

NUMBER	WATER	FAT	PROTEIN (N x 6.25)	ASH	WATER IN FAT-ASH-FREE SUBSTANCE	RATIO— PROTEIN TO WATER
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1	38.3	50.0	10.3	1.0	78.2	1 : 3.7
2	49.8	37.1	12.4	0.7	80.1	1 : 4.0
3	50.5	35.9	12.7	0.7	79.6	1 : 4.0
4	38.5	51.5	9.9	0.5	80.2	1 : 3.9
5	47.3	40.2	11.9	0.6	79.9	1 : 4.0
6	57.4	25.8	15.8	0.9	78.3	1 : 3.6
7	34.7	55.8	8.6	0.5	79.4	1 : 4.0
8	43.7	44.9	10.8	0.6	80.2	1 : 4.0
9	46.8	41.2	11.5	0.5	80.3	1 : 4.1
10	43.9	44.0	11.5	0.5	79.1	1 : 3.8
				Average	79.5	1 : 3.9

(B)—AS PREPARED FOR USE AT FOUR ESTABLISHMENTS OPERATING UNDER FEDERAL MEAT INSPECTION.

ESTABLISHMENT AND NUMBER	WATER	FAT	PROTEIN (N x 6.25)	ASH	WATER IN FAT-ASH-FREE SUBSTANCE	RATIO— PROTEIN TO WATER
A—1	41.9	46.8	10.6	0.6	79.7	1 : 4.0
2	50.0	36.6	12.8	0.7	79.7	1 : 3.9
3	51.2	34.6	13.2	0.7	79.1	1 : 3.9
B—1	49.8	35.3	13.2	1.2	78.4	1 : 3.8
2	42.7	44.8	11.5	0.9	78.6	1 : 3.7
3	48.5	38.4	12.4	0.7	79.6	1 : 3.9
C—1	39.7	49.5	10.2	0.6	79.6	1 : 3.9
2	47.5	38.6	13.3	0.7	78.3	1 : 3.6
D—1	49.6	37.5	12.3	0.6	80.1	1 : 4.0
2	44.2	42.6	12.7	0.6	77.8	1 : 3.5
				Average	79.1	1 : 3.8

18 grams of water, 2 grams of seasoning, and 5 grams of cereal were added to make Sausage B; and to 100 grams of Meat Mixture C, 20 grams of water and 2 grams of seasoning were added to make Sausage C.

TABLE 6.
Analyses of meat mixtures containing added water.

DESCRIPTION	WATER	FAT	PROTEIN (N x 6.25)	ASH	CEREAL	ADDED WATER IN SAUSAGE		WATER IN FAT-ASH- CEREAL- FREE SUB- STANCE
						By Analysis	By Cal- culation	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Meat Mixture A.	48.5	38.4	12.4	0.7				79.6
Meat Mixture B.	48.2	38.8	12.3	0.6				79.5
Meat Mixture C.	53.6	31.8	13.8	0.8				79.5
Sausage A.....	49.8	35.9	11.3	2.3	0.0	4.6	4.7	80.6
Sausage B.....	53.0	30.9	10.2	1.9	3.8	12.2	14.4	83.6
Sausage C.....	60.1	26.1	11.3	2.2	0.0	14.9	16.4	83.8

Applying the same deductions to the analyses of the experimental sausage mixtures, Table 6, the percentage of water (49.8) as determined in Sausage A exceeds four times the percentage of protein (11.3) by 4.6 per cent, the water actually added to Sausage A as calculated from the formulas being 4.7 per cent. In like manner the quantity of added water in Sausages B and C, as determined from the analyses, may be compared to the water actually added to the sausages as calculated from the formulas, with results as shown in Table 6.

From the examples cited it is evident that the quantity of added water determined by subtracting from the percentage of water found in the sausage four times the percentage of protein found, is approximately the same as the quantity actually added.

TABLE 7.

Comparison of manufacturing yields with analyses of Bologna Style Sausage.

	A	B	C	D	E
MANUFACTURING DATA.					
	lbs.	lbs.	lbs.	lbs.	lbs.
Beef and Salt	210	210	420	210	210
Pork Trimmings	90	90	180	90	90
Spices	3½	5½	11	3	3
Onions				2	2
Casings	29	21½	50	22	23
Total Raw Materials	332½	327	661	327	328
Ice Added	60	60	120	60	60
Total	392½	387	781	387	388
Meat Left in Pan After Stuffing		7	7	12	10
Stuffed Weight	396	380	774	375	378
Smoked Weight	366	350			
Cooked Weight	366	350	737	354	353
Chilled Weight	358	341	719	345	346
Less Materials Used	332½	320	654	315	318
Net Gain	25½	21	65	30	28
Percentage Gain	7.66	6.5	9.9	9.5	8.8
ANALYTICAL DATA.					
	per cent	per cent	per cent	per cent	per cent
Water Content	62.2	63.8	60.3	62.3	64.1
Protein (N x 6.25)	13.4	14.5	12.5	13.5	13.6
Added Water by Analysis	8.6	5.8	10.3	8.3	9.8
Variation from Manufacturing Record	0.9 more	0.7 less	0.4 more	1.2 less	1.0 more

To prove that the preceding statement is true of other types of sausage as well, the results of five tests made on Bologna Style Sausage as commercially prepared are given in Table 7. The manufacturing data were carefully checked by inspectors on duty at the establishment, which operates under Federal Meat Inspection. The analyses of the samples of finished sausage were made at the Meat Inspection Laboratory, Bureau of Animal Industry, Washington, D. C., and the analyst was not informed of the manufacturing data until he had reported his findings.

It may be seen from Table 7 that in the case of Bologna Style Sausage, prepared from both pork and beef, the quantity of added water, as determined by the proposed method of analysis, compares closely with the gain in weight due to water actually added. The results shown in Table 7 further indicate the adaptability of the analysis to sausages commercially prepared.

Tables 6 and 7 offer conclusive evidence that the determination of the quantity of added water in sausage by comparing the water content of the sausage with the normal water content of the meats therein, determined by the use of the protein-moisture ratio, is sufficiently accurate for all practical purposes.

Added moisture in sausage is detected and determined in the Meat Inspection Laboratory, Bureau of Animal Industry, by determining water and nitrogen according to the official methods of analysis¹. Protein is then calculated by multiplying the nitrogen content by the factor 6.25. The added water, if any, is next determined by subtracting from the moisture content, as found by analysis, the quantity of water naturally derived from the meats and meat by-products used as ingredients, as found by multiplying the protein content by the factor 4.0.

The Meat Inspection Laboratories of the Bureau of Animal Industry began, in 1914, to apply the 4 : 1 water-protein ratio to the detection and determination of added water in samples of uncooked and unsmoked sausage. As a result, the practice of adulterating sausage of this type with water was effectively checked in all establishments operating under Federal Meat Inspection. The introduction of a simple and effective laboratory method for the detection of added water made it possible to accomplish this result with less direct supervision than had previously been required, and thus effected a material saving in the expense of inspection.

Following the successful application of the 4 : 1 water-protein ratio to fresh sausage, attention was turned to the question of added water in smoked and cooked sausage, particularly Frankfurter Style and Bologna Style. In the manufacture of these sausages it is customary to add a considerable quantity of water to facilitate grinding, chopping, and mix-

¹ *Methods of Analysis*, A. O. A. C., 1925.

ing. Part of this added water is lost in subsequent processing, but the finished product generally contains more or less added water. The Meat Inspection Laboratories of the Bureau of Animal Industry therefore made an exhaustive survey of all brands and types of smoked and cooked sausage prepared in all establishments operating under Federal Meat Inspection to determine the quantity of added water found in the product as prepared under commercial operating conditions. The results of this survey showed the bulk of the finished product to contain less than 10 per cent of added water when shipped from the establishment to the trade. It also showed a tendency to abuse and excess on the part of a few establishments. In a series of 1,886 samples collected from inspected establishments throughout the United States, 400 were found to contain added water in excess of 10 per cent. Some individual samples contained as high as 31 per cent of added water.

Table 8 is illustrative of the abuse involved in the free and unrestricted incorporation of water in sausage. All four samples represent brands sold in the same market and at the same price to the consumer.

TABLE 8.

Comparative analysis of Frankfurter Style Sausage of same commercial grade and sold in same market.

ESTABLISHMENT	FAT	PROTEIN	WATER	ADDED WATER	CALORIES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per lb.</i>
A	15 8	13 7	66.8	12 0	895
B	26 7	14 9	54 3	0 0	1,365
C	30 9	15 0	49 0	0 0	1,530
D	27 9	13 9	53 9	0 0	1,390

The need for official regulation of the water content of smoked and cooked sausage having been demonstrated by this investigation, an amendment to the Meat Inspection Regulations was recommended by the Chief of the Bureau of Animal Industry and approved by the Secretary of Agriculture, whereby a limit of 10 per cent was set on the quantity of added water permitted in smoked and cooked sausage¹. Under authority of this amendment, which became effective October 1, 1925, the addition of water to smoked and cooked sausage in establishments operating under Federal Meat Inspection is now being satisfactorily controlled. The value and importance of this work is shown by the fact that the 771,741,200 pounds of sausage chopped under Federal Meat Inspection during the fiscal year ending June 30, 1926, was safeguarded from adulteration by application of the method described.

¹ Amend 3, Bur Animal Industry Order 211 (revised), 1925.

INFLUENCE OF SULFATES ON THE VOLUMETRIC METHOD FOR THE DETERMINATION OF PHOSPHORUS¹.

By C. M. BIBLE (Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh, Pa.).

Many chemists have experienced difficulty with the official volumetric method for the determination of phosphorus². Some still use the more time-consuming gravimetric method, because they do not have sufficient confidence in the volumetric procedure. Some that use the volumetric method make a practice of standardizing their alkali for titrations against a phosphate rock of known phosphorus content, because they find that results are too high if they use an alkali of theoretical strength, for example, forty-six molecules of alkali to one molecule of phosphorus pentoxide.

The writer believes that in many cases the failure of the official volumetric method to give accurate results is due to the influence of sulfates, either present in the original phosphatic material or introduced during the determination. The sulfates that cause a disturbance when the volumetric method is used apparently do no harm when the gravimetric method is used.

This conclusion was pointed out by Breckenridge³ in 1924. He found that the removal of sulfates by precipitation with barium nitrate enabled him to get results with the volumetric method that agreed closely with those obtained by the gravimetric method. In 1915 Falk and Sugiura⁴ found that ammonium phosphomolybdate, precipitated in the presence of sulfuric acid, contained sulfate, apparently as an essential part of the molecule.

Phosphate rock usually contains only a trace of sulfate, but when acidulated with sulfuric acid and converted into acid phosphate, the resulting material contains over 30 per cent of sulfur trioxide. Common mixtures of acid phosphate with sulfate of ammonia contain as much as 45 per cent.

Excessive amounts of sulfate are also present when sulfuric acid is used in getting the phosphorus into solution for analysis. Because certain phosphatic materials do not dissolve readily in nitric and hydrochloric acids, the official methods specify either (a) evaporation of the charge with a solution of magnesium nitrate, followed by ignition and solution in hydrochloric acid; or (b) digestion with nitric and sulfuric acids with occasional additions of sodium or potassium nitrate during digestion. For ease of manipulation in routine work, the writer has

¹ Presented before the Fertilizer Division at the Detroit Meeting of the American Chemical Society, September, 1927, and published here by courtesy of *Industrial and Engineering Chemistry*.

² *Methods of Analysis*, A. O. A. C., 1925, 31.

³ *Ind. Eng. Chem.*, 1924, 16: 1180.

⁴ *J. Am. Chem. Soc.*, 1915, 37: 1507.

found the latter method preferable to the ignition method. However, it introduces thirty or forty times as much sulfate as is usually present in the phosphate material.

Even the excessive sulfate contamination which results from a sulfuric acid digestion of the phosphatic material need not necessarily have a disturbing effect in the use of the volumetric method. Accurate results can be obtained without the sulfate removal by careful control of the temperature when precipitating the ammonium phosphomolybdate. The procedure that has given excellent results in the writer's laboratory is as follows: The aliquot of the phosphate solution is transferred to a beaker containing 50 cc. of 30 per cent ammonium nitrate solution, then neutralized with ammonia and made just acid with nitric acid. The solution is then heated to 45°C., but it is removed from the heating bath just before the ammonium molybdate is added. The mass is not heated after the addition of ammonium molybdate, but is stirred at room temperature for 30 minutes.

The results given in Table 1 are typical of what is obtained with different amounts of sulfate contamination and different conditions of precipitation of the ammonium phosphomolybdate. All the results were obtained at the same time, and none was omitted.

TABLE 1.

Phosphate rock with 34.40 per cent P_2O_5 content.

SAMPLE	METHOD OF PRECIPITATION OF AMMONIUM PHOSPHOMOLYBDATE		
	(a) Heat to 45°C.; add molybdate, stir 30 min at room temperature	(b) Keep at 45°C. for 30 min	(c) Keep at 65°C. for 15 min.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1 gram rock alone. Digested with nitric and hydrochloric acids	34.40 34.40	34.75 34.85	35.60 35.60
1 gram rock and 2 grams sulfate of ammonia. Digestion with nitric and hydrochloric acid	34.45 34.45	35.25 35.10	36.45 36.45
1 gram rock and 2 grams sulfate of ammonia. Digestion with nitric and sulfuric acids and sodium nitrate	34.45 34.40	35.40 35.60	36.40 36.40

In another series, samples were taken with different sulfur trioxide content, and the ammonium phosphomolybdate was precipitated by both methods (a) and (b) as given in Table 1 to find if the disagreement between the two methods of precipitation would be as great as that shown in the sulfate content.

TABLE 2.
Phosphatic material with varying SO₃ content.

DESCRIPTION	METHOD OF PRECIPITATION OF THE AMMONIUM PHOSPHOMOLYBDATE			
	SO ₃	(a) P ₂ O ₅	(b) P ₂ O ₅	Difference
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Phosphate rock	1	34.55	34.70	0.15
Check sample	8	39.60	40.10	0.50
Check sample	25	37.30	39.40	2.10

The writer's observations were not based on these few tests, however; they extend over a period of at least five years. A standard phosphate rock was run almost daily along with the routine work. When it was necessary to use sulfuric acid in the digestion of the routine samples, the phosphate rock standard was digested the same way, and the precipitation of ammonium phosphomolybdate was carried out as in modification (a).

The conclusion was drawn that carefully controlled conditions of precipitation of the ammonium phosphomolybdate is of the utmost importance if there is much sulfate present in the phosphate solution, either as the result of a sulfuric acid digestion of the material or owing to the presence of sulfates in the phosphatic material itself.

THE REDUCTION OF MAGNESIUM PYROPHOSPHATE BY CARBON.

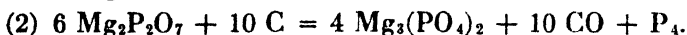
By K. D. JACOB and D. S. REYNOLDS (Bureau of Chemistry and Soils,
U. S. Department of Agriculture, Washington, D. C.).

When precipitates of magnesium ammonium phosphate are ignited in contact with the filter paper it is sometimes difficult to completely burn off the carbon. This trouble is encountered more frequently when dealing with large precipitates or when the ignitions are made over a gas burner than when dealing with small precipitates or when the ignitions are made in a muffle furnace. Ross¹ has shown that magnesium ammonium phosphate precipitates, when properly prepared and washed, will burn snow-white throughout when ignited in an electric muffle furnace for one hour at 1000°C. In this connection, the question that often arises—whether the filter paper carbon should be completely burned off at lower temperatures before finally raising the temperature to the maximum that is required—is an important one in view of the well-known fact that

¹ *This Journal*, 1927, 10: 190.

phosphates of the alkaline-earth metals may lose phosphorus when heated to high temperatures in contact with carbon. The reaction involves the reduction of the phosphorus-oxygen combination with evolution of elemental phosphorus.

In the case of magnesium pyrophosphate the reaction may be represented by either or both of the following equations:



Both of these reactions probably proceed simultaneously during the earlier stages of reduction.

In a search of the literature only one investigation dealing with the reaction between magnesium pyrophosphate and carbon came to the attention of the writers. The work reported in this investigation was carried out by Heraeus¹ in an effort to determine the cause of the deterioration of platinum crucibles used for the ignition of magnesium ammonium phosphate precipitates. He used the following procedure to determine the temperature at which the reaction between magnesium pyrophosphate and carbon begins.

A mixture of carbon and magnesium pyrophosphate, the latter having been ignited to constant weight, was placed in a glazed porcelain tube closed at one end, with an air-tight connection at the other end through a T-tube to a manometer and suction pump. This end of the porcelain tube also carried a calibrated thermocouple of special design, which was presumably placed in the interior of the mixture of carbon and magnesium pyrophosphate. The porcelain tube was connected in place in an electric furnace and repeatedly evacuated and filled with carbon dioxide until all the air was displaced. The tube was finally evacuated to a carbon dioxide pressure of about 7 cm. of mercury. It was then heated gradually, and the pressure and temperature were noted. These data were plotted, and the curve was compared with the curve obtained for the thermal expansion of carbon dioxide alone under identical conditions. Heraeus assumes that the break in the curve results from the increased pressure due to formation of carbon monoxide and phosphorus. His results indicate that the reaction between magnesium pyrophosphate and carbon begins at a temperature of about 950°C. However the data are purely qualitative, and no quantitative information on the reaction can be obtained from his experiments. Furthermore, there are three obvious sources of error in his experimental method.

In the first place, he has neglected to take into consideration the formation of carbon monoxide from carbon dioxide and carbon, as represented by the equation $\text{C} + \text{CO}_2 = 2 \text{CO}$. This reaction will result

¹ *Z. angew. Chem.*, 1902, 15: 917.

in an increase in pressure, and Kohn¹ has shown that it takes place rapidly above 900°C.

In the second place, Heraeus makes no statement as to the purity or method of preparation of his carbon. Lowry² has shown that some samples of carbon may contain as much as 0.5 per cent of hydrogen that is not driven off except by heating at temperatures above 900°C. These results have been confirmed by the writers on several samples of carbon from different sources. Both of these possible sources of error would contribute to an increase in pressure which, according to Heraeus' experimental method, would result in an apparently lower temperature of reduction of magnesium pyrophosphate than is actually the case.

In the last place, Heraeus determines his temperatures by means of a thermocouple that is presumably buried in the center of the mixture of magnesium pyrophosphate and carbon. In this event the temperature registered is not the highest temperature of the mixture at that particular instant, because magnesium pyrophosphate and carbon are both fairly good insulating materials.

The investigation described in this paper was undertaken to obtain quantitative data on the reduction of magnesium pyrophosphate at different temperatures under optimum conditions.

EXPERIMENTAL.

The magnesium pyrophosphate used in this work was prepared by igniting pure magnesium ammonium phosphate to constant weight at 900°-950°C. The latter salt was obtained by precipitating a solution of pure phosphoric acid with magnesia mixture according to the official gravimetric procedure for the determination of water-soluble phosphoric acid³. The magnesium pyrophosphate had the following composition:

	FOUND	THEORETICAL
	<i>per cent</i>	<i>per cent</i>
P ₂ O ₅	63 63	63 79
MgO.....	36 28	36 21
P ₂ O ₅	1 7538	1 7617
MgO		

For use in the experiments it was ground to pass an 80-mesh sieve.

The carbon used was a pure material prepared from petroleum coke. It contained 0.1 per cent of ash—practically all of which was silica, 98.7 per cent of fixed carbon, and 0.05 per cent of volatile matter at 900°C. It was ground to pass a 200-mesh sieve.

Three-gram mixtures containing equal parts by weight of carbon and magnesium pyrophosphate were used in all the experiments. Uniform

¹ *Ind. Eng. Chem.*, 1922, 14: 69.

² *J. Am. Chem. Soc.*, 1924, 46: 824.

³ *Methods of Analysis*, A. O. A. C., 1925, 4.

composition of the mixtures was obtained by agitating the materials for several hours in a bottle with a number of small rubber-covered lead balls. The prepared mixture was transferred to a graphite boat of such a size that the total thickness of the charge was about 3 mm. The boat was then placed in an electric resistance furnace of the graphite-tube type¹ and heated to the desired temperature in an atmosphere of nitrogen. Dry, oxygen-free nitrogen was passed through the furnace at the rate of about one liter per minute during each experiment. The boat was placed so that its back end was in the hottest portion of the furnace, thus assuring a gradient of temperature between the two ends of the boat. Overheating of the charge was also guarded against by the use of a false bottom in the graphite boat.

The temperatures of the front and the back of the boat were determined by means of a Leeds and Northrup optical pyrometer that had been standardized recently by the Bureau of Standards. The average of these two temperatures, which did not differ by more than 16°C., was taken as the temperature of the charge and was maintained within $\pm 5^\circ\text{C}.$ of the temperature desired. In all cases the necessary corrections for the deviation of the furnace from black-body conditions and for the absorption of light by the glass furnace window were applied to the temperatures as observed.

After the experiment was completed, the boat was pushed out of the hot zone by means of a rod working in a stuffing-box and allowed to cool in the furnace in an atmosphere of nitrogen. The residue was then carefully transferred to an agate mortar, ground to pass an 80-mesh sieve, and thoroughly mixed. In preparing the solutions for analysis, the weighed samples of the residues were first ignited in porcelain crucibles below 800°C. in order to burn off all the carbon. They were then dissolved in *aqua regia*². Each solution was diluted to 250 cc. and analyzed for magnesium oxide and phosphorus pentoxide, in both cases by precipitating as magnesium ammonium phosphate and weighing as magnesium pyrophosphate.

The percentage of phosphorus lost by volatilization was calculated from the change in the $\frac{\text{P}_2\text{O}_5}{\text{MgO}}$ ratio of the residue as compared with the ratio found for the original magnesium pyrophosphate. This relation is expressed by the simple formula $\frac{a-y}{a} = x$, where a is the $\frac{\text{P}_2\text{O}_5}{\text{MgO}}$ ratio of the original magnesium pyrophosphate, y is the $\frac{\text{P}_2\text{O}_5}{\text{MgO}}$ ratio of the residue, and x is the percentage of the total original phosphorus pentoxide that is volatilized.

The results of these experiments are given in the table. The data show

¹ Bryan, Mehring, and Ross, *Ind. Eng. Chem.*, 1924, 16: 821.

² When the residues were treated directly with *aqua regia* without first burning off the carbon, it was found that 0.5–1.0 per cent of the total P_2O_5 was adsorbed from solution by the finely divided carbon. Miller (*J. Phys. Chem.*, 1927, 31: 1197) has recently shown that positive adsorption of acids from solution is obtained with ash-free adsorbent charcoals.

that under the most favorable conditions only about 2.5 per cent of the total phosphorus originally present was volatilized when mixtures of magnesium pyrophosphate and carbon were heated at 1000°C. for 1 hour. About 5 per cent was volatilized in 2 hours under the same conditions. About 12.5 per cent of the phosphorus was volatilized in 1 hour at 1050°C. and about 32 per cent at 1100°C. The results indicate that about 0.25 to 0.60 per cent of the phosphorus was volatilized in 1 to 3 hours at temperatures of 900°-975°C. This loss is practically within the limit of experimental error, but it may be due in part to the presence of a very small quantity of magnesium metaphosphate which probably would be reduced at the lower temperatures. It was not determined whether or not the sample of magnesium pyrophosphate used in this investigation actually contained a small quantity of magnesium metaphosphate.

Effect of temperature and time of heating on the volatilization of phosphorus from mixtures of magnesium pyrophosphate and carbon.

(P_2O_5 - MgO ratio in original magnesium pyrophosphate = 1.7538)

TEMPERATURE	PERIOD OF HEATING	COMPOSITION OF RESIDUE			P_2O_5 VOLATILIZED— PART OF TOTAL ORIGINALLY PRESENT
		P_2O_5	MgO	P_2O_5 -MgO RATIO	
°C.	hours	per cent	per cent		per cent
900	1	33.68	19.30	1.7451	0.50
900	1	32.82	18.78	1.7476	0.35
925	1	32.10	18.35	1.7493	0.26
925	1	31.97	18.31	1.7460	0.44
925	3	32.01	18.36	1.7435	0.59
950	1	32.15	18.38	1.7492	0.26
950	3	32.14	18.42	1.7448	0.51
975	1	34.45	19.77	1.7425	0.64
975	1	33.98	19.48	1.7444	0.54
1000	1	31.48	18.32	1.7183	2.02
1000	1	31.60	18.59	1.6998	3.08
1000	2	31.74	19.02	1.6688	4.85
1050	1	29.35	19.32	1.5192	13.38
1050	1	29.66	19.25	1.5408	12.15
1100	1	25.67	21.47	1.1956	31.83

DETERMINATION OF CHLORINE IN BLEACHED FLOUR¹.

By ARMIN SEIDENBERG (Chemical Laboratory, Department of Health, New York, N. Y.).

Using the tentative methods for the determination of chlorine in bleached flour, Rask² and Bailey³ report results secured through collaborative work averaging between 16 and 32 parts per million for un-

¹ NOTE: This paper was submitted as an associate referee's report for the 1925 meeting and was referred to in the report of the General Referee on Cereal Foods at that meeting. Through an unfortunate oversight, publication was not made with the reports presented at the meeting.—F. C. Blanck, General Referee on Cereal Foods, 1927-28.

² This Journal, 1922, 6: 68.

³ Ibid., 1923, 7: 130.

bleached flour; for bleached flour, results showing a difference of as much as 100 parts per million were at times secured on the same sample. In some instances there was no sharp distinction between bleached and unbleached flour.

The writer has found the following conditions to be important:

- (1) Thorough extraction of the flour sample.
- (2) Removal of any natural chlorine from the ash, etc., carried along as an impurity.
- (3) Use of chlorine-free reagents.

The results obtained were practically negligible for unbleached flour, which was thus distinguished definitely from bleached flour.

The method used for the extraction of chlorine has been outlined by the writer¹, but it is given in this paper with numerous modifications and in greater detail.

By first treating the material with 70 per cent and then with 95 per cent ethanol it is possible to extract the added chlorine by the subsequent treatment with ethyl ether and petroleum ether. The extract contained in these solvents was washed twice with water in order to remove any natural chlorides that might have been carried along. By using the solvents in the proportions stated, which were determined after a series of experiments, it was found possible to avoid the formation of emulsions.

In the Mohr method for the determination of chlorine, titration with silver nitrate must be carried out in a neutral solution. Some of the alkali that is added to the solvent in order to hold the chlorine during the evaporation of the solvent reacts to form carbonates when the organic material in the residue is ashed. It is not possible to duplicate this condition in a blank because the extract is absent. As a result, different amounts of acid and alkali are required for neutralizing, and the actual quantity of chlorine in the reagents used for a determination cannot be accurately estimated. In the Volhard method², however, the same amounts of acid and alkali can be used in the blank as in the actual determination, because titration is conducted in an acid solution, and the chlorine content of the reagents used can be accurately calculated.

It has been found that potassium hydroxide or sodium hydroxide, even when labelled "C. P." or "Highest Purity", contains more or less chlorine. Metallic sodium, on the other hand, is entirely free from this impurity. When allowed to react with 95 per cent ethanol a reagent is secured that can be substituted to advantage for alcoholic potassium hydroxide. A clear alcoholic solution, which yields a better residue for ashing, is then obtained. Whereas 10 cc. of a 4 per

¹ *This Journal*, 1925, 8: 676.

² Treadwell and Hall. *Analytical Chemistry*, 6th ed 1924, II, 603; Sutton. *Volumetric Analysis*, 11th ed., 1924, 150 and 183.

cent alcoholic potassium hydroxide solution required approximately 6 cc. of 0.005 *N* silver nitrate, equivalent when calculated to 20 grams of flour to 53 parts of chlorine per million or as much chlorine as many samples of bleached flour have been found to contain, practically none was required for 10 cc. of the solution made with metallic sodium. Since the other reagents used contained comparatively little chlorine, a blank very low in chlorine was secured.

As will be noted from the table, duplicate determinations made on the same sample gave results that agreed within 6 to 7 parts per million. All the unbleached flours gave low results; inasmuch as they were within the experimental error of the method, they cannot be taken to indicate the presence of any chlorine. The bleached flour samples showed the presence of chlorine in quantities sufficient to distinguish them sharply from unbleached flour. In all cases, however, the amounts were less than those used in bleaching. It is notable that the two flours showed a consistent difference in this respect. The method is as follows:

REVISED METHOD FOR CHLORINE IN BLEACHED FLOUR.

REAGENTS.

- (a) *Ethanol*.—70 per cent by volume.
- (b) *Ethanol*.—95 per cent by volume.
- (c) *Ethyl ether*.
- (d) *Petroleum ether*.
- (e) *Alcoholic soda*.—To 95 per cent ethanol add metallic sodium cut into small pieces in the proportion of 40 grams of sodium to 1,000 grams of ethanol.
- (f) *1 : 3 nitric acid*.—Dilute 500 cc. of concentrated acid to 750 cc. with water.
- (g) *Silver nitrate solution*.—0.005 *N*.
- (h) *Potassium thiocyanate*.—0.005 *N*.
- (i) *Ferric-ammonium alum solution*.—To a cold saturated solution of ferric-ammonium alum add enough nitric acid to cause the disappearance of the brown color.

DETERMINATION.

Weigh 20 grams of flour into a 500 cc. Erlenmeyer flask, add 60 cc. of 70 per cent ethanol, place the flask upon a steam bath, and heat gently (water should steam but not boil), at the same time rotating the flask until the flour and liquid form a uniform mixture. Add 60 per cent of 95 per cent ethanol. Stopper the flask and shake thoroughly for 2 minutes. Allow to cool. Add 75 cc. of ethyl ether and shake the flask thoroughly; then add 150 cc. of petroleum ether and again shake the flask thoroughly. Pour the entire liquid contents into a separatory funnel, being careful to avoid, as far as possible, transference of any flour particles. Add to the flask containing the flour, 40 cc. of petroleum ether, shake thoroughly, and pour into the separatory funnel. Repeat with another 40 cc. portion of petroleum ether. Wash the solvents twice with water, using 30 cc. of water the first time and 10–12 cc. the second time; shake thoroughly each time, and allow to stand until two sharply defined layers of liquid are formed. Run the washed solvents into a large evaporating dish (or beaker), add 10 cc. of the alcoholic soda solution, and evaporate to about 10–15 cc. Pour this liquid into a 50 cc. platinum dish and wash out the evaporating dish with small portions of 95 per cent ethanol until all the liquid and residue have been transferred to the platinum dish. Evaporate the con-

tents of the dish to dryness on the steam bath and place the dish with the residue over a small yellow flame of a Bunsen burner. Char the residue but do not heat even to low redness because the alkali may react with the platinum. Allow to cool and add a small quantity of water and 5 cc. of 1 : 3 nitric acid. Boil, and then pour through an ashless filter paper (12½ cm. in diameter), catching the filtrate in a sugar flask calibrated at 100 cc. and 110 cc. Again boil residue with a small quantity of water and filter. Remove the filter paper, fold once and place in platinum dish, and heat to low redness until practically all of the paper and residue have been reduced to a gray ash. (Only a low heat may be used owing to the presence of chlorides.) Add a small quantity of water and 2½ cc. of nitric acid, boil, and filter. Again boil once or twice with small quantities of water, filtering as before. Add to the liquid in the sugar flask 25 cc. of 0.005 *N* silver nitrate solution, add water to bring the liquid approximately to the 100 cc. mark, and place the flask in boiling water for about 5 minutes. Remove, and allow to cool to room temperature. Bring the liquid exactly to the 110 cc. mark by adding water, stopper the flask, and mix the contents well. Filter through a dry, fine-pore filter paper (12½ cm. in diameter), and return the first portion of the filtrate to the original solution. Continue to refilter until the filtrate is entirely clear, and thus secure 100 cc. of filtrate. Transfer the entire 100 cc. to a white porcelain casserole, add 2 cc. of ferric-ammonium alum solution, and titrate with 0.005 *N* potassium thiocyanate until a permanent light brown coloration appears. Deduct the blank determined on all the reagents used and calculate results to the dry basis.

CALCULATION.

$$25 - (1.1 \times \text{No. cc. KCNS}) \times 0.0001775 \times 50,000 = \text{chlorine (parts per million)}.$$

Chlorine determinations.*

(Made by associate referee.)

SAMPLE NO.	CHARACTER OF SAMPLE	PARTS PER MILLION	
		Quantity Applied	Quantity Determined
UNBLEACHED SAMPLES			
1	Clear	None	0
2	Patent.	None	4
3	Winter Patent...	None	0
4	2nd Clear.	None	0
5	Canadian Wheat (Hard Spring).	None	3
6	Hard Patent	None	0
7	Winter Wheat (Straight)	None	3
8	Blended Clear.	None	4
		Average	1.8
BLEACHED SAMPLES			
1	Clear....	50	31
			24
		150	106
			101
			28
			104
2	Patent	50	25
			18
		150	76
			70
			22
			73

* Blank equals 3 parts per million.

METHOD FOR ESTIMATING FIELD CORN IN CANNED MIXTURES OF FIELD AND SWEET CORN.

By JOHN L. HEID¹ (Skinner Manufacturing Co., Omaha, Nebr.).

In an investigation of sweet and waxy-sweet corn made at the Ohio State University, Lampe and Meyers² reported a new kind of cell content in the immature endosperm, with the following description: "It is a globule of cytoplasmic origin which stains red with iodine. As these globules increase in size, smaller grains of solid carbohydrate are usually found within them".

Although the identity of the carbohydrate is undetermined, the color reactions obtained by Lampe and Meyers were previously reported by Weatherwax³ and Kempton⁴. Culpepper and Magoon⁵ also reported that "the sweet varieties contain a high percentage of water-soluble polysaccharides which appear to consist of dextrin-like substances mixed with varying amounts of material similar to soluble starch".

The grains of solid carbohydrate in ordinary (non-waxy) sweet corn are starch and stain blue with iodine.

Collaboration with Lampe and Meyers led to the formulation of a method for the estimation of field corn in canned mixtures of field and sweet corn at the U. S. Food and Drug Inspection Station in Cincinnati. G. P. Larrick of that station and P. L. Gowen of the Food Control Laboratory, Bureau of Chemistry, Washington, D. C., collaborated extensively with the writer in this work, and determinations were made at other stations as shown in the tables.

This method depends upon the fact that sweet corn contains red-staining (iodine) erythro dextrin, while field corn does not. Examination consists of testing the interior of separate kernel fragments for the presence of dextrin and calculating the percentage of each type of corn in the mixture.

PROCEDURE.

The entire content of the can is emptied into a large beaker, and the liquor and debris are removed from the fragments of kernels by flotation, cold water being used. All kernel fragments to which the seed coat is still attached are then placed upon a flat plate, thoroughly mixed, and quartered to about 400 pieces. The pieces are hardened for several hours in 95 per cent alcohol and again quartered to obtain a subdivision of about 100 kernel fragments for examination. Each of the fragments is cut through with a section razor, or knife, the instruments being im-

¹ The work reported in this paper was done while the writer was connected with the U. S. Food and Drug Inspection Station, Cincinnati, Ohio. He is especially grateful for the valuable assistance given by the collaborators.

² *Science*, 1925, 61: 290.

³ *Genetics*, 1922, 7: 568.

⁴ *Genetics*, 1923, 57: 566.

⁵ *J. Agr. Res.*, 1924, 28: 423.

mersed in water and wiped dry each time to prevent the transference of dextrin to subsequently examined kernels. A portion of the kernel about one-sixteenth of an inch in diameter is removed from the center with a dissecting needle and placed in a separate depression upon a white porcelain spot plate. (The portion is taken from the interior of the kernel to insure against previous impregnation with dextrin from the can liquor.)

Each fragment of endosperm is covered with iodine stain (0.2 gram iodine, 1.5 grams potassium iodide in 100 mls. of water) and allowed to stand 10 minutes. A dense brown cloud will disseminate from the particles of sweet corn, while the solution surrounding the field corn particles will be clear and the particles will be blue-black and sharply outlined. The field corn particles are then crushed with the needle to make certain that they contain no dextrin. Those found to contain no dextrin are counted, and the percentage of field corn is calculated from the total number examined.

COOPERATIVE STUDY.

In order to determine the accuracy of the method and its practicability in the hands of analysts not familiar with its technique, authentic packs containing known mixtures of field and sweet corn were prepared. Samples were submitted without indication of the quantity of field corn which had been added. Table 1 lists the data submitted by the Bureau of Chemistry. Table 2 shows data obtained from a pack prepared by the Cincinnati Station. Comments received from the collaborating analysts assisted in clarifying the details of the procedure so as to insure accurate and uniform results.

TABLE 1.
Authentic pack prepared by P. L. Gowen.
(Results expressed in percentage.)

SAMPLE NUMBER	FIELD CORN ADDED	FIELD CORN FOUND BY COLLABORATORS					
		P. L. Gowen	J. L. Heid,	L. Mitchell, St. Louis	L. Mitchell (tips only)	A. B. Vaughn, New York	H. W. Smith, Baltimore
3C	50	52	51	55	51 7	56	38
4C	25	30	24	23 2	29 2	23 2	24
5C	10	11	15	8 4	13 9	18 2	21
6C	5	4	8	3 3	5 6	6 2	6

SAMPLE NUMBER	FIELD CORN ADDED	S. C. Rowe, Philadelphia	FIELD CORN FOUND BY COLLABORATORS				Chernoff, Denver
			L. Jones, Chicago	Haynes and Elliot, Boston	Callaway, Savannah	Feldstein, Denver	
3C	50	48	38.7	48	18	49	39
4C	25	27	33 3	22	26	26	30
5C	10	13	14 7	24	15	27	39
6C	5	6	1.33	6	8	13	33

TABLE 2.
Authentic pack prepared by G. P. Larrick.
 (Results expressed in percentage.)

SAMPLE NUMBER	FIELD CORN ADDED	FIELD CORN FOUND	
		P. L. Gowen	J. L. Heid
1	10	12	14
2	20	19	19
3	40	38	42
4	50	39	47

SUMMARY.

It will be noted from the tabulations that fairly concordant results were obtained in most instances. Widely divergent results were obtained by some of the collaborators, but the comments accompanying the reports indicate that these discrepancies were attributable to the difficulty in obtaining uniform samples and not to the interpretation of the method. The examination of authentic packs indicates that the method is of value in detecting intentional adulteration of canned sweet corn with field corn.

NOTE.

Note on the Sand-Gooch Method for Analysis of Butter.—In the sand-gooch continuous extraction method proposed by Mitchell and Alfend¹ a certain river sand found near Minneapolis, Minnesota, is used. This is a drawback to the method owing to the difficulty of obtaining the sand and to the special treatment to which it must be subjected.

It has been found that 90-mesh crystalline alumina (R. R. Alundum. The Norton Co., Worcester, Mass., blue label) is a satisfactory substitute for sand for the following reasons: It is a uniform product, easily obtainable; it needs no purification or preparation; its ability to hold the butterfat during drying is equal to that of the sand; the butterfat is removed completely by the continuous extraction with carbon tetrachloride; the alumina is not attached by the butter; it has no tendency to cake or fuse during ignition or ashing, even at a bright red heat; and its price is nominal.

The substitution of crystalline alumina for the Minneapolis sand will make this method more desirable for general use.

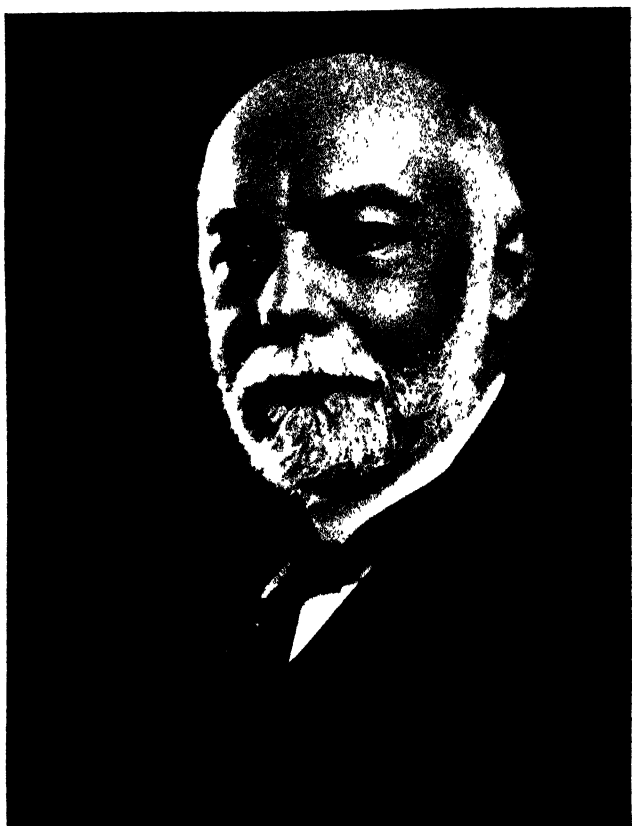
L. C. MITCHELL,
Food, Drug and Insecticide Administration, St. Louis, Mo.

¹ *This Journal*, 1926, 9: 209.

JOINT CONFERENCE.

Four hundred printed and bound copies of the papers presented at the joint meeting of the Association of Official Agricultural Chemists, Association of Dairy, Food and Drug Officials, and Association of Feed Control Officials held in Washington, D. C., October 20, 1926, in commemoration of the 20th anniversary of the passage of the Federal food and drugs act are available for distribution to members and subscribers who request them so long as this supply lasts.

W. W. SKINNER, *Secretary-Treasurer*,
Box 290, Pa. Ave. Station, Washington, D. C.



FREDERICK BELDING POWER, 1853-1927

FREDERICK BELDING POWER

In the death of Dr. F. B. Power the Association of Official Agricultural Chemists lost one of its most distinguished and amiable members. Although Dr. Power's relations with this society did not begin until he was sixty-seven years of age, he was a constant attendant at all the annual meetings from 1920 to 1926, and his papers and discussions contributed greatly to the work of the association. Members and visitors recall with pleasure his attractive and genial personality for he had a natural faculty of forming friendships.

Frederick Belding Power was born on March 4, 1853, in Hudson, New York, the son of Thomas and Caroline Belding Power. His early education was derived at a private school and at the Hudson Academy, which he was obliged to leave at the early age of 13 in order to begin the task of earning a livelihood. He found employment in the local drug store of Mr. Whitfield, where he worked for the next five years. During this period he not only became familiar with all the technical operations of a country drug store, but he also acquired a considerable knowledge of chemistry, gained mostly from the reading of Dr. Edward Parrish's "Treatise on Pharmacy". His indulgent employer gave some direction to the studies of the boy and permitted him to make experiments with such chemicals as his stock afforded, although the stench which emanated from some of these operations had at times the unhappy consequence of driving the proprietor and customers from the store.

In 1871 the young pharmacist accepted a position as assistant in the Chicago pharmacy of Thomas Whitfield, a brother of the Hudson apothecary. He lived in a room located over the pharmacy of his employer at the corner of State and Van Buren streets, but he had been here only a few months when the famous fire of October 8-10, 1871, wiped out almost the entire business and residential sections of Chicago. At this time he spent two whole days on the lake shore without food and water until the progress of the flames had been stayed and assistance could be brought to the refugees.

The following year Power decided to resign his position in Chicago and seek employment in Philadelphia, where he hoped to advance his chemical and pharmaceutical knowledge at the Philadelphia College of Pharmacy. Upon arriving in Philadelphia, with only 25 cents in his pocket, Power applied first to Dr. Edward Parrish, to whom he had a letter of introduction and whose name he had long known as the author of the "Treatise on Pharmacy", which had served as his first guide to the subject. Dr. Parrish offered him a position in his establishment and made arrangements for his matriculation at the College of Pharmacy. Parrish's sudden death might have caused a disarrangement of these plans, had not other influential friends at the college, such as John M. Maisch, Professor of Materia Medica and Botany, William Proctor, Jr., Professor of the Theory and Practice of Pharmacy, and Robert Bridges, Professor of Chemistry, taken an active interest in the young man.

Power completed his course at the Philadelphia College of Pharmacy in 1874 in the same class with his friend Henry S. Wellcome, but he con-

tinued his connection with the Parrish Company until 1876, when he determined to carry out the long cherished plan of completing his chemical and pharmaceutical education in Germany. He selected Strassburg as the university which offered the best courses in his favorite studies, and here he studied until 1880 under such teachers as Fittig and Friedrich Rose in chemistry, Flückiger in pharmacognosy, Schmiedeberg in pharmacology, DuBary in botany, and Kund in physics. His favorite professor was Flückiger, who made him his private assistant in 1879-1880.

In the autumn of 1880 Power returned to America to accept the newly established professorship of analytical chemistry at the Philadelphia College of Pharmacy, a position which he retained until 1883. During this period, in collaboration with his friend, Dr. Frederick Hoffmann of New York, he prepared his "Manual of Chemical Analysis", which was written primarily for students of pharmacy.

In 1883 President Bascom of the University of Wisconsin invited Power to organize the Wisconsin School of Pharmacy with the title of professor and dean, and the offer was gladly accepted. During this connection he contributed twenty articles upon essential oils, alkaloids, and other plant principles. In 1884 he translated Flückiger's monograph on the cinchona barks and in 1887 Flückiger and Tschirch's "Principles of Pharmacognosy". He was a member of the Committee of Revision of the U. S. Pharmacopeia for 1890 and rendered distinguished service both in this and in the later revisions.

Power resigned his position as Dean of the Wisconsin School of Pharmacy in 1892, in order to accept a position as the scientific director of the newly-established technical laboratories of Fritzsche Brothers at Passaic in New Jersey. A variety of articles upon oils of cloves, peppermint, bay, wintergreen, sweet birch, sassafras and other essential oils was contributed to the *Pharmazeutische Rundschau* and the *Pharmaceutical Review* during his connection with this laboratory. He also prepared a "Descriptive Catalogue of Essential Oils and Organic Chemicals", which was published by Fritzsche Brothers in 1894.

Power's classmate, Henry S. Wellcome, established in 1896 the Wellcome Chemical Research Laboratories at 6 King Street, London, of which he tendered the directorship to Power, who gladly accepted the offer. His eighteen years' residence in London was the most productive period of his life. The papers of which he was either sole or co-author numbered 75, and they constitute a record of accomplishment in pharmaceutical and plant chemistry that is almost without parallel. He often worked until after midnight upon the preparation of manuscripts or the perusal of phytochemical literature. In this period he made exhaustive investigations of the constituents of over 50 different plant products with a great extension of the existing knowledge upon the distribution of different organic compounds in the vegetable world.

The first results of Power's most widely known research, upon the constituents of chaulmoogra seeds, were made known in 1904. This long investigation culminated in his famous paper upon the constitution of chaulmoogric and hydnocarpic acids, published in the *Journal of the Chemical Society* in 1907. This research is a classic from which all future investigators of the leprosy-healing derivatives of chaulmoogra oil must begin as their starting point.

In 1900 Power published his suggestions relating to the revision of the

British Pharmacopeia which led to results as important as those previously accomplished by him in the revision of the United States Pharmacopeia.

Honors were showered upon Power from all parts of the world during his London residence. In 1903 he won for the second time, and in 1907 for the third time, the Ebert Prize of the American Pharmaceutical Association. Gold medals were conferred upon him at the St. Louis International Exhibition of 1904, at the Milan Exhibition of 1906, at the Franco-British Exhibition of 1906 in London, and at the Turin Exhibition of 1911. A silver medal was conferred upon him at the Liège Exhibition of 1905 and a Grand Prize at the Brussels Exhibition of 1910. The Chemical, Linnaean, and Pharmaceutical Societies of Great Britain jointly awarded him the Hamburg Gold Medal in recognition of his distinguished researches upon the natural history and chemistry of drugs. In 1908 the honorary degree of doctor of laws was conferred upon him by the University of Wisconsin and in 1913 the Philadelphia College of Pharmacy conferred upon him the honorary degree of master in pharmacy.

Conditions resulting from the outbreak of the World War in 1914 caused a complete interruption of Power's research work at the Wellcome Chemical Laboratories. He returned to the United States in 1915 and accepted a position in Washington as head of the newly constituted phytochemical laboratory of the Bureau of Chemistry in the United States Department of Agriculture, beginning his work on May 23, 1916.

In the eleven years of his connection with the Bureau of Chemistry, Power published seventeen papers, his research work during this period being conducted in collaboration with V. K. Chesnut, who was a co-author in most of the contributions. These papers relate principally to *Ilex vomitoria* as a native source of caffeine, to a chemical examination of the tuberous roots of *Cyperus esculentus*, to the odorous compounds of apples, peaches and grapes, and to the volatile and non-volatile constituents of the cotton plant. In point of completeness Power regarded the last-named research, which was his final laboratory investigation, as his most finished piece of work.

At the 1920 meeting of the Association of Official Agricultural Chemists Dr. Power presented a most important paper¹ upon "The Detection of Methyl Anthranilate in Fruit Juices", an analytical research which has been of the greatest benefit in the examination of grape and other beverages. The method worked out by Dr. Power and his colleague, Mr. Chesnut, for determining caffeine² in coffee and tea was also reported upon at this meeting and adopted as an official method of the association. Members of the association will also recall Dr. Power's interesting discussions³ upon monobromated camphor and chaulmoogra oil at the 1924 meeting.

Several additional honors were conferred upon Dr. Power during the final Washington period of his interesting chemical career. In May, 1921, he received a gold medal, bearing his profile in relief, from his life-long friend Henry Wellcome. In 1922 he was awarded the Flückiger Gold Medal by the Swiss Pharmaceutical Society. It is doubtful if any award ever brought him greater pleasure than the medal which bore the name and likeness of his beloved teacher. In 1924 Dr. Power was elected a member of the National Academy of Sciences, and on April 27, 1925, he presented before that body his last scientific paper, which was upon the odorous constituents of the cotton plant.

¹ *This Journal*, 1921, 5: 225; *J. Am. Chem. Soc.*, 1921, 43: 377.

² *Ibid.*, 271, 291.

³ *Ibid.*, 1925, 8: 514, 525.

After completing sixty years of active life in pharmacy and chemistry, Dr. Power decided to discontinue all further work in the laboratory and to devote the remainder of his days to writing a comprehensive two-volume treatise upon phytochemistry. He had prepared the general scheme of his subject and had just begun to fill in the outline when he was suddenly called from the activities of this earthly life on the morning of March 26, 1927. Funeral services were held on March 29 in Washington and also on April 1 at his birthplace in Hudson, New York, where his remains were buried.

Dr. Power was a member of the National Academy of Sciences, the American Chemical Society, the Washington Academy of Sciences, a fellow of the American Association for the Advancement of Science, and a corresponding member of the Royal Society of Pharmacy of Brussels. He was delegate from the United States to the International Congress for Unification of the Formulae of Potent Medicaments at Brussels in 1902, a vice-president of the Society of Chemical Industry in 1904-6, a president of the Washington Chemical Society, and first vice-president of the United States Pharmacopeial Convention of 1920.

Dr. Power was a leading member of the Church of the Covenant in Washington, of which he was made an elder on March 27, 1924. His religious convictions were firmly grounded. He did not obtrude his views upon others, yet he was always glad in the presence of sympathetic listeners to discuss the great questions of life and immortality and to renew the expressions of his own hope in a Great Hereafter. The spiritual attitude of Frederick Power, the unraveller of so many mysteries of plant life, is perhaps best expressed in the immortal lines of Tennyson,

“Flower in the crannied wall,
I pluck you out of the crannies,
I hold you here, root and all, in my hand,
Little flower—but if I could understand
What you are, root and all, and all in all,
I should know what God and man is.”

C. A. BROWNE.

FIRST DAY.

MONDAY—MORNING SESSION.

No report on waters, brine, and salt was given by the referee.

No report on tanning materials and leathers was given by the referee.

REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. GRAHAM (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

During 1927 the referee continued the study of methods for the determination of unsulfonated residue in mineral oils. The associate referee appointed at the last meeting studied the methods of analysis for the determination of fluorine compounds and did considerable work, which he will present in a separate report.

The report on the work of the referee follows:

The study of methods for the determination of unsulfonated residue in mineral oils was continued.

Four samples of oil were sent to collaborators with the following directions:

METHODS FOR THE DETERMINATION OF UNSULFONATED RESIDUE IN MINERAL OILS.

Method I.

This method has been published¹.

Method II.

With a pipet measure 5 cc. of the oil into a Babcock cream bottle about 15 cm. (6 inches) long, either the 9 gram 50 per cent or the 18 gram 30 per cent type. (With heavy oils, to reduce the viscosity, warm the pipet after a preliminary draining by drawing it several times through the flame of a Bunsen burner and then drain thoroughly.) In lieu of measuring, determine the specific gravity of the oil and weigh the equivalent of 5 cc. into the bottle. Add slowly 20 cc. of the 37 *N* acid in four equal portions, shaking after each addition. Take care that the temperature of the mixture does not rise above 60°C. and cool in ice water if necessary. When the mixture no longer develops heat on shaking, agitate thoroughly, place the bottle in a water bath, and heat at 60°–65°C., holding this temperature for 1 hour and shaking for a period of 20 seconds at 10 minute intervals. Remove the bottle from the bath and fill with concentrated sulfuric acid until the oil rises into the graduated neck. Centrifugalize for 5 minutes (or longer if necessary to obtain a constant volume of the oil) at 1200–1500 revolutions per minute

¹ *This Journal*, 1927, 10: 30.

TABLE
Collaborative results—
(Expressed

ANALYST	SAMPLE 1					
	Method I		Method II		Method III	
	Vol.	Wt.	Vol.	Wt.	Vol.	Wt.
John W. Elmore Department of Agriculture Sacramento, Calif.	76.3	76.8	82.2	82.7	80.3*	80.2*
	76.3	76.3	81.9	88.0	80.2*	79.9*
Average	76.3	76.6	82.1	82.4	80.3	80.1
H. J. Fisher Agricultural Experiment Station New Haven, Conn.	70.0	73.1	78.4	80.2	80.8	81.9
	72.0	73.9	78.8	80.2	78.4	80.6
Average	71.0	73.5	78.6	80.2	79.6	81.3
J. J. T. Graham	75.2	75.4	80.0	79.8	78.4	78.5
	74.4	74.6	78.8	78.7	78.4	78.2
Average	74.8	75.0	79.4	79.3	78.4	78.4
E. L. Green Agricultural Experiment Station Pullman, Wash.		74.2		81.6		
		73.8		81.6		79.6
		75.6		82.7		80.8
		75.7				
Average		74.8		82.0		80.2
E. L. Griffin Food, Drug and Insecticide Administration Washington, D. C.	76.0	76.2	82.0	81.8	81.2	80.9
	75.6	76.1	81.2	81.3	80.8	80.6
Average	75.8	76.2	81.6	81.6	81.0	80.2
H. Heidenhain Wenatchee, Wash.	76.0	77.0	80.0	81.1	81.0	81.4
	75.0	76.4	81.0	81.6	80.0	80.9
Average	75.5	76.7	80.5	81.4	80.5	81.2
R. R. McKibbin MacDonald College, Canada	79.6		82.4			
	76.8		82.8			
Average	78.2		82.6			
J. B. Terry Standard Oil Co. of California Richmond, Calif.	75.0	75.5	80.0	80.8	79.0	80.5
	73.0	73.6	80.0	81.5	80.0	81.0
	73.0	74.0	80.0	81.5	79.0	80.0
Average	73.3	74.4	80.0	81.3	79.3	80.5
General Average	74.9	75.2	80.6	81.2	79.8	80.3

* Oil column dense and black, necessitating the addition of water to facilitate the reading.

1.

unsulfonated residue in mineral oil.

in percentage.)

SAMPLE 2						SAMPLE 4					
Method I		Method II		Method III		Method I		Method II		Method III	
Vol.	Wt.	Vol.	Wt.	Vol.	Wt.	Vol.	Wt.	Vol.	Wt.	Vol.	Wt.
62.6	62.6	73.9*	73.7*	71.4*	71.2*	72.0	72.5	78.7*	79.4*	75.8*	76.8*
62.6	63.5	73.5*	74.2*	70.7*	70.6*	72.5	73.0	79.4*	79.3*	76.2*	77.1*
62.6	63.1	73.7	74.0	71.1	70.9	72.3	72.8	79.1	79.4	76.0	77.0
62.4*	63.5*	70.8*	73.2*	71.2*	71.7*	70.0*	73.4*	77.6*	79.7*	76.0*	78.4*
60.0*	63.9*	72.8*	74.4*	71.4*	72.0*	72.0*	73.8*	74.8*	79.7*	75.8*	78.4*
61.2	63.7	71.8	73.8	71.3	71.9	71.0	73.6	76.2	79.7	75.9	78.4
63.6	64.4	70.8*	71.0*	68.8*	68.5*	70.4*	71.2*	75.2*	76.2*	74.0*	74.3*
64.0	64.2	70.0*	71.1*	69.2*	69.4*	70.4*	71.6*	75.2*	76.0*	73.6*	74.1*
63.8	64.3	70.4	71.1	69.0	69.0	70.4	71.4	75.2	76.1	73.8	74.2
	62.7		71.0*		68.7*		71.4*		Could not read		76.6*
	62.5		70.4*		70.1*		70.9*				76.0*
	62.6		70.7		69.4		71.6				76.3
64.8	64.8	71.6*	71.7*	71.6*	71.7*	73.2*	73.4*	76.8*	77.1*	76.8*	76.9*
64.8	64.7	72.4*	72.6*	71.6*	71.8*	74.4*	74.6*	77.6*	78.1*	76.8*	77.1*
64.8	64.8	72.0	72.1	71.6	71.8	73.8	74.0	77.2	77.6	76.8	77.0
67.0	68.1	70.0*	71.3*	70.0*	71.1*	67.0*	67.2*	77.0*	77.9*	76.0*	77.4*
66.0	67.6	70.0*	71.2*	70.0*	71.0*	71.0*	71.1*	77.0*	77.6*	76.0*	77.2*
66.5	67.9	70.0	71.3	70.0	71.1	69.0	69.2	77.0	77.8	76.0	77.3
69.2*		70.8*				74.8*		76.4*			
66.8*		71.2*				74.8*		76.8*			
68.0		71.0				74.8		76.6			
68.0	68.0	71.0	71.8	68.0	69.0	69.0	70.5	77.0	78.4	74.0	76.0
66.0	66.8	70.0	71.2	68.0	68.8	69.0	70.3	76.0	77.5	74.0	75.9
67.0	67.1	70.0	71.5	68.0	69.6	69.0	70.2	76.0	77.6	75.0	76.3
67.0	67.3	70.3	71.5	68.0	69.1	69.0	70.3	76.3	77.8	74.3	76.1
65.0	65.0	71.3	72.0	70.0	70.3	71.3	71.7	76.8	78.0	75.4	76.6

and cool to 25°C. Read the volume of the unsulfonated residue from the graduations on the neck of the bottle and, to convert to cubic centimeters, multiply the reading from the 9 gram 50 per cent bottle by 0.1 and that from the 18 gram 30 per cent bottle by 0.2. From the result thus obtained calculate the percentage by volume of the unsulfonated oil.

Method III.

Proceed as in Method II except to heat the bottles at a temperature of 100°C.

NOTES FOR THE ANALYST.

It is the desire of the referee that in the work this year all charges for sulfonation be measured. In this connection it is important that the pipet be heated thoroughly and that sufficient time be allowed for the pipet to deliver the full charge of oil. With these precautions there should be no difficulty in measuring accurately the charge of oil. As a check on the accuracy of this method of apportioning the charges, weigh the oil in the bottles before sulfonation; from this weight and the specific gravity calculate the volume of oil used in each sulfonation and report results for unsulfonated residue on the basis of volumes of oil both by weight and measure.

In some sulfonations no line of demarcation is visible between the oil and the acid. If a reading cannot be made by the aid of a strong light, it will be necessary to add a few drops of water to form a layer.

Full comment and criticism of the methods is important and will be appreciated. In the work last year it was observed that oils that gave an opaque oil column with Methods II and III often gave a clear oil column with Method I. Information on this point is especially desired this year. The referee would also like to know which sulfonations required the use of water to facilitate the reading.

The collaborative results are given in Table 1.

DISCUSSION OF RESULTS.

Before discussing the results reported this year it will be well to review briefly those obtained last year. The samples sent out at that time were two spray oils from California and an Eastern red engine oil. These samples were sulfonated by the methods used in this year's work and also by another method in which 37 N acid at 65°C. was used for a period of 18 minutes and they were shaken for 20 seconds at 3 minute intervals. This last method was numbered 3 and Method 3, 1927, was numbered 4 in the 1926 work.

Last year a marked difference in the behavior of the oils when sulfonated was noted. Samples 1 and 2 gave clear oil columns in all cases. Sample 3 gave clear oil columns in most cases with Methods I and III, but with Methods II and IV, in which the sulfonation was continued for an hour, the oil columns were so contaminated with a black, tar-like, polymerization product that the volume could not be read without the addition of a few drops of water to form a layer between the acid and the column of oil. In such instances the oil volume found was greater than when a clear column was obtained.

In view of these facts it was the belief of the referee that Methods II and IV were undesirable and that Method I was the best of all those tested.

In planning the work for this year it was the intention of the referee to use one sample that could be easily handled by any of the methods, and in order to give the methods a severe test, to use for the other samples oils having a tendency to form the tar-like polymerization product. Samples 2, 3, and 4 are lubricating oils and are examples of the latter class, but in the actual collaborative tests Samples 3 and 4 proved to be more difficult to handle than had been anticipated. In fact, Sample 3 proved so unsatisfactory for the work that the results have not been included in the table. It is only fair to state that Samples 3 and 4 would probably never be used for spray purposes, and that they were only included in the work for the purpose of giving the methods an extreme test.

An examination of the values of unsulfonated residue reported by the analysts and given in Table 1 shows that there is little choice between the methods so far as consistency of the results is concerned. Although the results do not check so closely as might be desired, they appear in a better light when it is considered that the methods used were empirical and were formulated for the purpose of giving an index to certain properties of the oils and not for the purpose of determining their constituents exactly.

Commenting upon the methods, Elmore states that Method I gave a good column with all of the oils. Method II gave a good column with sample No. 1, but the other oils gave such dark columns by this method that it was necessary to raise the columns with water before they could be read. Method III gave dark columns with all the oils, and therefore all were raised to facilitate the reading.

Fisher expressed the opinion that there is little choice in the methods from the point of view of reproducibility of results. He believes that Method I is to be preferred because it requires less time and because it gives lower results, that is, the sulfonation is more complete.

Green does not like Method III because of the necessity of working at the high temperature required, and he believes that the results vary too little from those obtained at 65°C. to justify the added discomfort. He used Method I on approximately 30 oils in the course of an oil spray project and found it satisfactory.

Griffin prefers Method I because the time required is less, because it gives a clear reading on oil No. 2—whereas the other methods do not—and because 38 *N* sulfuric acid is required in another A. O. A. C. sulfonation method and therefore is a stock acid in many laboratories. He has used Method I in the analysis of several hundred lubricating oils used for spray purposes, from all the principal regions in which these oil emulsions are used, and he believes that this method will give a clear reading more often than either of the other methods.

Griffin prefers to determine the results by volume rather than by weight. He states that in none of his collaborative work did the actual volume of the oil, as determined by weight and density, vary more than 0.03 cc. from 5 cc., and that this accuracy is acceptable for a method in which the results are dependent on the readings of a Babcock bottle.

Terry states that he prefers Method III for the reason that consistent results have been obtained by this method in his laboratories, as well as in those of the various State Departments of Agriculture and of spray oil manufacturers throughout the whole Pacific Slope. He also states in this connection that practically no oils having an unsulfonated residue below 60 per cent are permitted in that territory.

Heidenhain states that in his opinion the method of measuring the oil is good enough for all practical purposes although he found the volumes of the oil delivered by the pipet to be short in every instance. However, he considers that the error of this measurement would not seriously affect the result so far as the characterization of the oil is concerned.

Of the 8 analysts participating in the work, four preferred Method I, one preferred Method III, and the remaining three expressed no preference.

The fact that Methods II and III give a dense, black oil column, necessitating the addition of water for the reading, more often than does Method I, is a strong point in favor of Method I. These readings are always higher than the true sulfonation value. This error is probably due to suspended colloidal material formed by polymerization during the long heating in the presence of the strong acid.

Method I, with a slight verbal change, is again recommended for adoption as an official method. The third sentence should be changed to read as follows:

If greater accuracy is desired, the measured charge may be weighed and its exact volume calculated from the weight and specific gravity of the oil.

This change in the wording of the method will save considerable time for those who prefer to weigh their charges of oil for the sulfonation, because it relieves them of the time-consuming operation of weighing the equivalent of exactly 5 cc. and substitutes in its stead the weighing of the quantity of oil delivered by a 5 cc. pipet.

RECOMMENDATIONS¹.

It is recommended—

(1) That in Method I for the determination of unsulfonated residue in mineral oils and in the recovered oil obtained in the analysis of oil-soap emulsions, the following sentence be substituted for the third sentence: "If greater accuracy is desired, the measured charge may be

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 64.

weighed and its exact volume calculated from the weight and specific gravity of the oil"; and that the method then be adopted as an official method (final action).

(2) That the methods for the determination of cyanogen and chlorine in sodium and potassium cyanides¹ be dropped as official methods (final action).

(3) That the method for the determination of cyanogen in sodium and potassium cyanides² be adopted as an official method (final action).

(4) That Methods I and II for the determination of chlorine in sodium and potassium cyanides³ be adopted as official methods (final action).

(5) That the method for the determination of cyanogen in calcium cyanide⁴ be adopted as an official method (final action).

(6) That Methods I and II for the determination of chlorine in calcium cyanide⁴ be adopted as official methods (final action).

(7) That the method for the determination of moisture in soap⁵ be dropped as an official method (final action).

(8) That the xylene distillation method for the determination of water in soap⁶ be adopted as an official method (final action).

(9) That the methods for the determination of water, total oil, and ash in mineral oil-soap emulsions⁶ be adopted as official methods (final action).

(10) That the method for the determination of soap in mineral oil-soap emulsions⁶ be adopted as an official method (final action).

REPORT ON FLUORINE COMPOUNDS.

By G. A. SHUEY⁷ (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*.

The list of insecticides mentioned in *Methods of Analysis* does not include fluorine compounds. It has been recognized, of course, that sodium fluosilicate has certain insecticidal properties, but it has never been advocated for use in the field. The fluosilicates were practically unknown as poison compounds for insects, although a patent was granted to an Englishman, C. H. Higbee (Patent No. 8236), about thirty years ago for their use as a poison for rodents. For many years sodium fluoride has been well known as a poison for roaches and chicken lice.

The work of Marcovitch⁸ has proved beyond all doubt the efficacy of fluosilicates as insecticides. These substances are now being used exten-

¹ *Methods of Analysis*, A. O. A. C., 1925, 65.

² *This Journal*, 1927, 10: 27.

³ *Ibid.*, 28.

⁴ *Ibid.*, 29.

⁵ *Methods of Analysis*, A. O. A. C., 1925, 65.

⁶ *This Journal*, 1926, 9: 28.

⁷ Presented by J. J. T. Graham.

⁸ Tenn. Agr. Exp. Sta. Bull. 131, 1924

sively in the control of the Mexican bean beetle and in combating the blister beetle in the rice section of southwest Louisiana, and further experimentation indicates a more extended use of them and of other fluorine compounds, singly and in combination, as insecticides.

With the increasing use of these salts it is obvious that methods for their analysis are necessary. Therefore an effort has been made to provide such methods.

The associate referee worked with several of the methods used for analyzing fluorine to ascertain their adaptability for the analysis of fluosilicates. For agricultural purposes sodium fluosilicate and so-called calcium fluosilicate compounds, which are by-products in the manufacture of acid phosphate, have largely been used. However, certain impurities, such as silica, air-slaked lime, calcium carbonate, phosphates, iron, aluminum, and traces of other salts find their way into by-product fluosilicates, and in some cases—and this is especially true in regard to silica and lime—foreign substances may be added directly.

Some of these impurities preclude the use of certain analytical methods, and frequently make necessary preliminary testing and treatment of the samples. Therefore, a number of samples were collected, and preliminary treatments are being studied with the object of standardizing details of procedure before submitting samples for collaborative study.

Of the numerous methods that have been proposed for the determination of fluorine, four show promise of being applicable to the analysis of fluosilicates. Each of these has received some attention in this work.

The first method is that of Berzelius¹, as modified by Rose² and by Treadwell and Koch³. In this method the sample is fused with sodium and potassium carbonates; the silica is then precipitated with ammonium carbonate and ammoniacal zinc oxide; and phosphates, if present, are removed as silver phosphate. Then calcium fluoride and calcium carbonate are precipitated by the addition of an excess of calcium chloride. The mixed precipitates are then ignited, calcium carbonate is dissolved in dilute acetic acid, and calcium fluoride is weighed. This method gives low results owing to the solubility of calcium fluoride in water and acetic acid.

The second method, that of Schucht and Möller⁴, prescribes that the fluosilicate salt be treated with an excess of neutral calcium chloride solution and then titrated with 0.1 *N* sodium hydroxide solution, phenolphthalein being used as an indicator. There is also another method, in which an excess of standard alkali is added to a solution of the fluosilicate sample; the mixture is then titrated with standard acid, a suitable indicator, usually phenolphthalein, being used. These volumetric

¹ Pogg. *Ann.*, 1824, 1: 1, 169; Schweigger. *Jour.*, 1816, 16: 426.

² Liebig. *Ann.*, 1849, 72: 343.

³ *Z. anal. Chem.*, 1904, 43: 469.

⁴ *Ber.*, 1906, 39: 3693.

methods give fairly accurate results with pure fluosilicates. Difficulty is encountered, however, in obtaining a titration end point that is distinct. When impurities such as traces of alkalies, sodium carbonate, or lime are present, preliminary treatment of the sample is necessary to prevent reaction with the fluosilicate salt.

The third method, and the one that has received the most attention in this work, is the so-called "volatilization" method. It was proposed by Offermann¹, investigated by Wagner and Ross², and further improved by Reynolds, Ross, and Jacob (see p. 225). This method is based on the principle that fluorine is volatilized as silicon tetrafluoride when a fluosilicate is intimately mixed with silica and heated with concentrated sulfuric acid. The silicon tetrafluoride thus formed may be passed into water and the resulting hydrofluosilicic acid titrated with standard alkali, phenolphthalein being used as an indicator. When the improved apparatus of Reynolds, Ross, and Jacob is used, fairly concordant results have been obtained by this method. The results, however, are slightly lower than the theoretical yield, even with pure fluorides. Because of its rapidity and accuracy, and also its applicability to the analysis of the numerous fluorine-bearing compounds that are gaining prominence as insecticides, this method merits further investigation. To improve the method the associate referee has designed a flask which permits the introduction of sulfuric acid without removal of the stopper and thus prevents the entrance of atmospheric moisture. The flask may be heated directly by the burner flame with little danger of breakage, since there are no sealed-in tubes at the heated part of the flask. Some attention has also been given to different types of tubes for absorbing the silicon tetrafluoride at the end of the train.

The fourth method that has been studied is that of Steiger³ and Merwin⁴. In this method the color produced by the action of hydrogen peroxide or titanium sulfate is bleached by fluorides. The colors are then compared against a standard. Several trials indicate that the method merits further consideration in regard to its adaptability for the analysis of fluosilicates.

Data have been obtained on each of the methods discussed, but they are not sufficient to be included in this report. It is hoped, however, to get collaborative work under way very soon⁵.

¹ *Z. angew. Chem.*, 1890, (3): 615.

² *J. Ind. Eng. Chem.*, 1917, 9: 1116.

³ *J. Am. Chem. Soc.*, 1908, 30: 219.

⁴ *Am. J. Sci.*, 1909, 28: 119.

⁵ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 65.

REPORT ON SOILS AND LIMING MATERIALS.

By W. H. MACINTIRE¹ (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Referee*.

The scope of soil investigations by this association was broadened during the past year by the appointment of an associate referee to study methods for the determination of less common metals in soils, and by the separate consideration of acid and alkali soils in studies of reaction values. Further studies have been directed toward the perfection of the technic and apparatus used for the determination of the causticity of limes. This work has resulted in the preparation of two technical papers which are given in the report of the Associate Referee for Liming Materials.

RECOMMENDATIONS.

It is recommended—

(1) That the work done by the Associate Referee for Less Common Metals in Soils serve as the basis for further work during the coming year.

(2) That the report of the Associate Referee for Liming Materials be adopted.

No report on the reaction value of alkaline soils was given by the associate referee.

REPORT ON REACTION VALUE OF ACID SOILS.

By E. T. WHERRY (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The determination of the reaction values of soils is a subject of such complexity and at the same time of such recent development that as yet even tentative methods cannot be definitely recommended. It nevertheless seems worth while to draw up a brief summary of the present status of the various methods available.

The hydrogen electrode method has the advantage of giving a direct reading of the potential due to hydrogen ion (the source of acidity) and of being capable of high precision. It has, however, the disadvantage that its operation is difficult and time-consuming and extreme precautions have to be taken to avoid introducing errors, especially with respect to contamination of the hydrogen, poisoning of the electrode,

¹ Presented by W. W. Skinner.

and varying the temperature. Moreover, in soils part of the acidity is due to carbon dioxide, and this is driven out by the entering hydrogen, so that a correction must be applied when the original reaction value is desired. In a recent study, Olsen and Linderström-Lang, in Denmark¹, first endeavored to retain the carbon dioxide by the use of a special (Hasselbalch) electrode vessel, but they found that the results could not be depended upon, so they obtained a correction factor by measuring with the quinhydrone electrode the hydrogen-ion potentials of two portions of a suspension of the soil, one receiving no special treatment, and the other having had a current of carbon-dioxide-free air passed through it for 24 hours. The difference between the two readings was then subtracted from the hydrogen-electrode reading, obtained in the usual way. The correction values thus derived show, however, unduly wide fluctuations from one soil to another, even being negative in some cases (that is, driving out the carbon dioxide appeared to increase the acidity). It accordingly seems necessary to conclude that it is at present impracticable to obtain precise measurements of soil reaction with the hydrogen electrode, at any rate not closer than ± 0.5 pH unit.

The quinhydrone method is quicker and easier of operation than the preceding, and is less subject to disturbances. It cannot be used very far on the alkaline side of the neutral point, however, and appears to be subject to serious error in soils containing relatively large amounts of inorganic colloids, especially when rich in ferric oxide. Some authors have found essential agreement between the two electrometric methods in the majority of soils, while others, including Olsen and Linderström-Lang, claim some disagreement, the quinhydrone method yielding in general lower acidities. There being no evidence that this method is markedly less precise than the other, its simplicity certainly recommends it for routine work.

The colorimetric method has the advantage over any electrometric method of requiring far simpler apparatus and less manipulation, but it too has inherent disadvantages. Many workers prefer to filter the soil-water mixture and make the observations on the more or less clear extract obtained, cancelling the effect of any residual turbidity and of color by superimposing a layer of the extract on the color standard. In the experience of the writer any filtration procedure, using paper or porcelain, is likely to change the reaction markedly, although the colodion sac method as worked out by Pierre and Parker² does not produce appreciable change. However, by using observation cells with parallel walls set but 4 or 5 mm. apart, and the usual arrangement for cancellation of turbidity or color, satisfactory readings can be obtained in practically every case after an hour's settling and filtration is not neces-

¹ *Compt.-rend. trav. lab. Carlsberg*, 1927, 17 (1): 27 pp

² *Soil Sci.*, 1927, 23: 13-32.

sary. Olsen and Linderström-Lang find evidence that the sulfonaphthal-ein indicators give results consistently 0.35 pH unit more acid than the hydrogen electrode, although between pH 5 and 8 the loss of carbon dioxide during the filtration process tends to make the readings as much as 0.4 pH unit less acid. These minor errors inherent in the indicator method seem insufficient to offset its advantages, and it may be depended upon for measurements of soil reaction under all ordinary circumstances to ± 0.5 pH unit.

The questions as to how much water to add to facilitate reaction measurement, and how long the soil-water mixture should be allowed to stand before being worked upon, require little consideration. Different workers have obtained widely divergent results from varying the soil-water ratio, and until the reason for the discrepancies can be ascertained, all that can be recommended is that the minimum amount of water be added. As to the time of standing, it is urged that determinations be made as soon as practicable after adding the water to the soil, at most an hour or two being permitted to elapse. The reason for this is that the addition of a considerable excess of liquid favors both increased interaction between soil particles and more active growth of micro-organisms, resulting in reaction values different from those originally represented in the soil.

When those for whom the results of hydrogen-ion determination are intended can think in logarithms, and when the relation between a phenomenon under study to the reaction is exponential, then pH values are appropriately used. In the experience of the writer the majority of laymen and not a few scientists find it impossible to grasp the significance of logarithmic numbers, and, moreover, there is evidence that the growth of plants, in connection with which subject most work on soil reaction is done, is related more directly to the hydrogen-ion concentration than to any exponentially derived values. Accordingly, for the purposes of making the results widely intelligible and of enabling conclusions as to the relationships between plant growth and soil reaction to be correctly drawn, it is recommended that in general soil reactions be stated not in exponential pH values but in some sort of terms expressing the hydrogen-ion and hydroxyl-ion concentrations¹.

REPORT ON LIMING MATERIALS.

By W. M. SHAW² (Agricultural Experiment Station, Knoxville, Tenn.),
Associate Referee.

The work of the associate referee for the past two years was directed along two lines: (1) The perfection of the technic of the tentative official

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 65.

² Presented by W. W. Skinner.

method for the determination of the so-called available lime; and (2) the effect of impurities on the accuracy of the results obtained. As a result of such efforts, a simple filtration device that greatly improves the technic and the accuracy of the modified sugar method was evolved. The fact was established that impurities in a lime sample will cause variations in the results that are in proportion to the time of contact during the determination.

Because of the interest of the lime industries in the subject matter, it was thought advisable to publish the findings and conclusions in an industrial journal¹.

The following method was formulated:

DETERMINATION OF THE CAUSTIC VALUE OF LIME.

APPARATUS.

A filtration device as shown in the figure. In this apparatus (*A*) is a 500 cc. Erlenmeyer flask of Pyrex glass, and (*F*) is a filter cone packed nearly full with cotton. The cotton is covered to a depth of from 2 to 3 mm. with lightly compacted macerated filter paper. The filter cone is connected with the syphon tube (*B*) by means of thick-walled rubber tubing. The receiving flasks (*m*) and (*n*) are calibrated to deliver 50 and 100 cc., respectively. (*S*) is a suction flask.

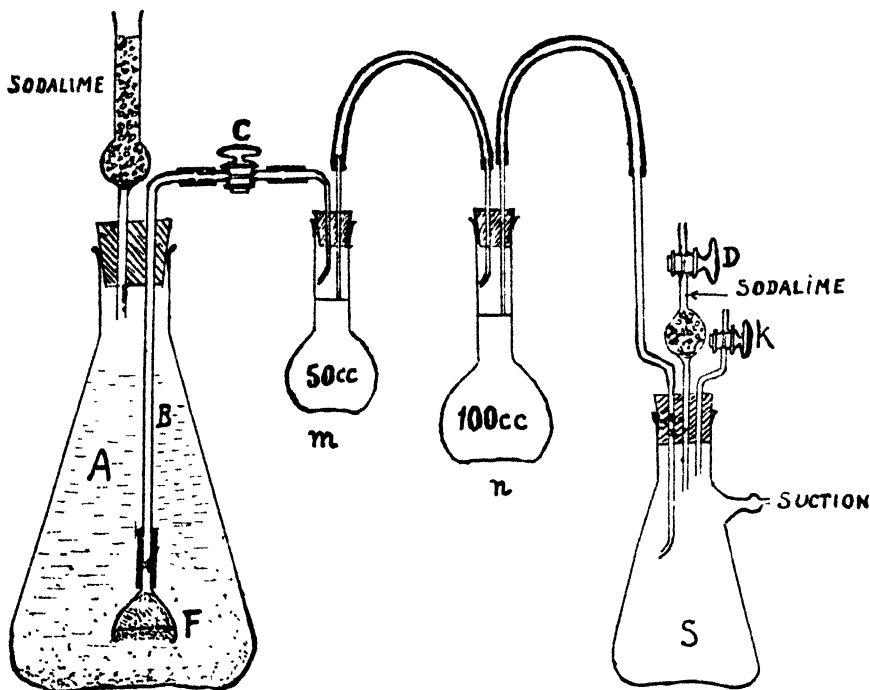


FIG. 1.—APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS.

¹ *Ind. Eng. Chem.*, 1928, 20: 312.

DETERMINATION.

Grind the lime sample to pass a 100-mesh sieve, mix thoroughly by rolling, and place in a sample container. (Prepare the sample in an atmosphere of minimum moisture and carbon-dioxide content and protect it from the breath of the operator.) Transfer a portion of the sample to a weighing bottle. By means of a polished, narrow-pointed spatula that has been calibrated to hold approximately 1.5 grams, withdraw the charge to be used and determine its exact weight by difference. Introduce the charge directly into the dry flask (A) provided with a tightly fitting, rubber stopper.

Prepare a sugar solution *immediately before use* by placing 25 grams of granulated sugar in a measuring flask calibrated to deliver 500 cc. Dissolve the sugar with cold carbon-dioxide-free water and make up to the mark. Hold both the Erlenmeyer flask containing the charge and the flask containing the sugar solution in a slightly inclined position, insert the neck of the sugar solution flask a short distance into the Erlenmeyer flask, and carefully transfer the sugar solution while simultaneously and synchronously agitating both flasks by a rotary motion to prevent granulation of the lime. Stopper the Erlenmeyer flask securely and agitate. Add, if desired, a quantity of clean dry beads. Effect complete solution of uncoated caustic lime by six 1-minute agitations at intervals of 2 or 3 minutes. Crush any undisintegrated particles of the sample by careful twisting of the stopper after inverting the flask to trap them in the space between the stopper and the neck of the flask. Allow 15 minutes further contact between the lime and the sugar solution, and then filter.

Connect the filter cone (F) with the syphon (B) and close stopcock (D). Connect the receiving flasks, apply suction, and quickly connect the Erlenmeyer flask (A) containing the lime solution with the stopper (E). Open stopcock (C) and filter 25 to 50 cc. of the solution. Close (C) and open (D) to release suction. Remove (m) and replace with another dry flask of the same kind. Close (D), open (C), and continue the filtration until both (m) and (n) have been filled at least to the marks. To disconnect the system, close stopcock (C), press the outlet of the flask (m) down gently and then the outlet of the flask (n) to remove any excess of liquid above the marks. Permit the intermediate connection to empty, and then open stopcock (D) and remove (m) and (n). Titrate the first 50 cc., or pilot aliquot, of the filtered solution with 0.5 N hydrochloric acid, using phenolphthalein indicator. Run twice the volume of the 0.5 N acid required for this titration into a covered 200 cc. beaker, add the second, or 100 cc. aliquot, of the filtered solution to this acid and phenolphthalein indicator, and complete the titration.

Calculate the caustic value of the sample by means of the formula:

$$X = \frac{7A}{W}, \text{ in which}$$

X = percentage of active calcium oxide;

A = cc. of 0.5 N acid used per 100 cc. of lime solution; and

W = weight of charge.

RECOMMENDATIONS¹.

It is recommended—

(1) That the sugar method as described by the associate referee be adopted as an official method for the determination of the caustic value of lime.

(2) That Methods I and II² be deleted from *Methods of Analysis*.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 65.

² *Methods of Analysis*, A. O. A. C., 1925, 5, 6.

No report on less common metals in soils was given by the associate referee.

REPORT ON FEEDING STUFFS.

By W. F. STERLING (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

The Associate Referee on Stock Feed Adulteration devised and studied collaboratively a method for the detection of dried buttermilk in feeds. The method depends upon the detection of lactic acid bacilli, which seem to be a diagnostic characteristic of the dried milk product. The results obtained justify the recommendation that the method be adopted as tentative.

The Associate Referee on Mineral Mixed Feeds directed collaborative study on methods for the determination of calcium oxide and iodine in feeds. He recommends that further work be done on these methods as written and that certain modifications be studied.

The Associate Referee on Moisture did preliminary work on a method that requires that the sample be heated at a temperature above that of boiling water and at atmospheric pressure. By this method a number of samples could be run simultaneously; it would also provide an official method that could be used under conditions where vacuum apparatus is not available. The Referee on Feeding Stuffs recommends that the method be submitted to collaborative study and that the samples submitted include a molasses feed and a linseed meal.

The referee concurs in the recommendations of the associate referees and suggests that all the lines of work pursued this year be continued.

REPORT ON STOCK FEED ADULTERATION.

By H. E. GENSLER (Department of Agriculture, Harrisburg, Pa.),
Associate Referee.

The microscopical detection of dried buttermilk in feeding stuffs has been one of the problems of the microanalyst. The difficulty lies in the fact that when viewed under the microscope, buttermilk shows no characteristic tissue or cell structure. The associate referee resorted to colorimetric qualitative tests for the detection of lactic acid, which is a constant ingredient of dried buttermilk, and later gave some consideration to the determination of lactose, which might also be considered an index of its presence. However, since lactic acid and lactose are merely *indications* of dried buttermilk, it was necessary to find a method that would determine buttermilk itself.

In the method presented to the collaborators advantage was taken of the fact that dried buttermilk invariably contains numerous lactic acid

bacilli which, by the application of an appropriate stain, are made visible under the microscope.

The method is as follows:

MICROSCOPICAL DETECTION OF DRIED BUTTERMILK IN FEEDING STUFFS.

Mount in water, upon a clean glass slide, about 5 mg. of that portion of the feed which passes through a 40-mesh sieve. Spread the material uniformly and keep for 5-10 minutes upon a level surface in a warm place to dry. When dry, immerse the slide in xylol or gasoline for 1 minute, and dry again. Next immerse the slide in 90 per cent alcohol for at least 1 minute and then in a fresh aqueous solution of methylene blue, allowing it to remain in the latter solution from 5-60 seconds. Rinse the slide in water and decolorize in alcohol for several seconds, watching so that decolorization does not proceed too far. Dry and examine with the microscope, using a 1.9 mm. (1/12 in.) oil-immersion objective without a cover glass. If areas containing many blue-stained bacilli are found, dried buttermilk is present.

Five samples of feeding stuffs containing different amounts of dried buttermilk were presented to the collaborators for analysis. In addition they were requested to examine other buttermilk samples in order to ascertain whether or not the lactic acid bacillus is always present in dried buttermilk and to report concerning the presence of these bacilli in feeds in which there was no buttermilk. The reports submitted indicate that the lactic acid bacillus is practically always present in buttermilk and that it is not likely to be found in feeds to which dried buttermilk has not been added.

The results obtained by the analysts in the application of the method are given in the table. They indicate that the method itself is reliable. In only one case was buttermilk reported in sample No. 1. In samples Nos. 2, 3, 4, and 5, which contained 3, 0.50, 1, and 7 per cent of buttermilk, respectively, incorrect reports were submitted in comparatively few cases.

The results obtained and the opinions of the collaborators strongly indicate that the method submitted is a practical and reliable procedure for detecting dried buttermilk in feeds. Any error may be attributed to the personal factor of the analyst. Therefore, it is recommended that this method for the detection of dried buttermilk in feeding stuffs be adopted as a tentative method¹.

Results obtained in the detection of dried buttermilk.

ANALYST	SAMPLE NO. 1 NO BUTTERMILK	SAMPLE NO. 2 3.00 %	SAMPLE NO. 3 0.50 %	SAMPLE NO. 4 1.00 %	SAMPLE NO. 5 7.00 %
1	None	Present	None	Present	Present
2	"	"	Present	"	"
3	Trace	"	"	"	"
4	None	"	"	"	"
5	"	None	"	"	"
6	"	Present	"	"	"
7	"	"	"	"	"
8	"	"	None	"	None
9	"	None	Present	"	Present

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 66.

REPORT ON MINERAL MIXED FEEDS.

By H. A. HALVORSON (State Dairy and Food Department, St. Paul, Minn.), *Associate Referee*.

At the last meeting of this association, the Associate Referee on Mineral Mixed Feeds was instructed to continue the study of the proposed methods for the determination of lime (CaO) and iodine in mineral mixtures and to submit samples to collaborators for analysis.

Two samples of known composition, resembling commercial mixed feeds, were sent to fourteen collaborators who had indicated a willingness to assist in this work. Of this number, eleven collaborators reported results of the determination of lime (CaO) in both samples, and eight reported results of their determinations of iodine by the proposed method.

Sample No. 1 was made up of the following ingredients: tankage 10 per cent, spent bone black 35 per cent, ground limestone 35 per cent, salt (sodium chloride C. P.) 19.9 per cent, potassium iodide 0.1 per cent. Determinations of the calcium oxide in each ingredient by the proposed method showed that the limestone furnished to the mixture 19.06 per cent, the spent bone black 17.06 per cent, and the tankage 0.88 per cent, making a total of 37.00 per cent. Assuming that the method used was satisfactory and the individual determinations correct, and also that there was no calcium oxide in the salt and potassium iodide, the exact or theoretical percentage of calcium oxide in sample No. 1 was 37.00 per cent. By calculation from the percentage of potassium iodide used, the iodine in this sample was 0.0764 per cent.

Sample No. 2 was made by mixing 40 per cent tri-basic calcium phosphate (pure precipitated), 40 per cent calcium carbonate (precipitated), 19.95 per cent salt (sodium chloride C. P.), and 0.05 per cent potassium iodide. When calcium oxide was determined in two of the ingredients, as described previously, it was found that the calcium phosphate furnished to this mixture 19.83 per cent and the calcium carbonate 22.03 per cent of calcium oxide. The total calcium oxide in this sample, therefore, should be 41.86 per cent. Calculated from the amount of potassium iodide used, the iodine in this sample should be 0.0382 per cent. The method submitted to the collaborators for the determination of lime (CaO) in the samples was published in the report of the associate referee last year¹.

Table 1 gives the individual results and the averages submitted by eleven collaborators. A study of these results shows them to be fairly satisfactory when the nature of the product and the unfamiliarity with the method on the part of some of the collaborators are taken into con-

¹ *This Journal*, 1927, 10: 177.

sideration. Experience has shown that better results can be obtained by more practice with the method and by checking up the standard solutions used. Most of the collaborators expressed satisfaction with the method and were in sympathy with further work on it. Several suggested other methods for study, and two recommended the use of acetic acid in place of hydrochloric acid at the point where the solution is made neutral. It was pointed out that if acetic acid was used, the precipitation could be made in the acid solution and it would not be necessary to use the time to make the solution exactly neutral with 0.1 *N* sodium hydroxide. In the opinion of the associate referee, this modification should be tried next year in order to compare the results with those obtained by the method as originally proposed.

TABLE 1.
Calcium oxide in A. O. A. C. mineral feed samples.

COLLABORATORS	SAMPLE NO. 1.—37.00 % CALCULATED FROM ANALYSIS OF INGREDIENTS		SAMPLE NO. 2.—41.86 % CALCULATED FROM ANALYSIS OF INGREDIENTS	
	Individual	Average	Individual	Average
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
J. M. Bartlett Orono, Me.	38.02 38.44 38.30	38.25	42.51 42.60 42.51	42.54
G. S. Fraps College Station, Tex.		38.60		43.50
J. W. Kellogg Harrisburg, Pa.		37.71		43.69
E. L. Redfern Des Moines, Ia.		39.06		43.26
A. W. Clark Geneva, N. Y.		36.84		41.98
L. E. Bopst College Park, Md.		37.31		42.01
W. B. Griem Madison, Wis.	37.25 37.31	37.28	42.12 41.92	42.02
H. R. Kraybill Lafayette, Ind.	37.19 37.34 37.34	37.29	42.20 41.91 42.48	42.20
W. F. Hand A. and M. College, Miss.		38.05		42.26
Howell D. Spears Lexington, Ky.	{ 37.45 37.66 37.94 37.66	37.56 37.80	41.02 41.44 41.86 41.44	41.23 41.65
A. O. Olson St. Paul, Minn.		37.00		41.69

The collaborators were also instructed to determine iodine in the two samples by the proposed method as published in the report of the associate referee last year¹, also to modify the method by using carbon tetrachloride instead of carbon disulfide at all points where carbon disulfide is mentioned. In this modification 25 cc. of a very concentrated ferric chloride solution was used as the oxidizing agent instead of hydrogen peroxide. Instructions were also given for preliminary treatment of the samples, as follows: If the sample contains organic matter such as tankage, oil meal, etc., in addition to the regular mineral constituents, the sample should be charred slightly in a muffle, but no attempt should be made to get the sample white or to destroy the black material which forms. Extreme care should be exercised to prevent any volatilization of the iodine. The purpose of this treatment is to facilitate the extraction of any iodine that may be held by fats, oils, or organic ingredients.

TABLE 2.
Iodine in A. O. A. C. mineral feed samples.

COLLABORATOR	SAMPLE NO. 1.—0.0764 % ADDED		SAMPLE NO. 2.—0.0382 % ADDED	
	Carbon Disulfide Method	Carbon Tetrachloride Method	Carbon Disulfide Method	Carbon Tetrachloride Method
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
J. M. Bartlett	0.0481		0.0399	
G. S. Fraps	0.0450		0.0220	
J. W. Kellogg			0.0370	
W. B. Griem	0.0514	0.0390	0.0343	0.0272
H. R. Kraybill	0.0510		0.0190	
W. F. Hand	0.0700	0.0300	0.0450	0.0240
Howell D. Spears	0.0610	0.0620	0.0331	0.0440
A. O. Olson	0.0400		0.0120	

Table 2 shows the averages of the iodine results obtained by eight collaborators. Some of the collaborators expressed dissatisfaction with the proposed method and strongly recommended that the ferric chloride and carbon tetrachloride modification be not considered; two expressed complete satisfaction with the method when carbon disulfide and hydrogen peroxide were used. While in most cases the results reported are considerably below the amounts added to the sample, they are nevertheless, in the opinion of the associate referee, sufficiently accurate to war-

¹ *This Journal*, 1927, 10: 176.

rant additional work on the proposed method. Attempts to determine iodine in mineral feeds heretofore have almost invariably proved disappointing. Therefore, any method that enables the analyst to report definite quantities of iodine should be considered and further studied in the hope that the difficulties encountered may be overcome. Other methods for the determination of iodine in mineral feeds have been suggested and should be tried out in comparison with this method, but there seems to be no doubt of the advisability of dropping further work on the carbon tetrachloride and ferric chloride modification.

RECOMMENDATIONS¹.

It is recommended—

(1) That the proposed method for the determination of lime (CaO) in mineral feeds be further studied and that the acetic acid modification of this method be tried for comparison of results.

(2) That the proposed method for the determination of iodine in mineral feeds published last year be further studied and that consideration also be given to other proposed methods.

REPORT ON MOISTURE IN FEEDING STUFFS².

By G. E. GRATTAN (Department of Agriculture, Ottawa, Can.), *Associate Referee*.

Reference to the literature shows numerous and varied methods for the determination of moisture in feeding stuffs; most of these, however have some drawback to make them impracticable for routine laboratory work when several hundred samples must be examined in a short space of time.

The vacuum oven method, while admittedly the most accurate, entails the use of a costly piece of apparatus which a large number of the State laboratories are unable to obtain. The use of a current of dry hydrogen does not seem to be popular. Mechanical difficulties, together with the lack of any instruction as to how the hydrogen is to be obtained or dried, present themselves. Drying in vacuum without heat is lengthy and tedious and requires too much apparatus. The toluene distillation method, while accurate and fairly rapid, requires considerable apparatus which on the whole is expensive. The task of keeping the apparatus "chemically clean" takes time and must be considered.

A method then is desirable from the point of view of economy, speed, and ease of manipulation—one that will produce results in close harmony with those obtained by any other standard or official method.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 66.

² *Ibid.*

Before undertaking any work, a general survey was made of the various State laboratories in order to learn what apparatus was available and what method was being employed. A questionnaire was sent to forty-six laboratories, and replies were received from thirty-four. They are enlightening and may be summarized as follows:

<i>Vacuum oven method.</i> —Temperature 65°–105°C.; time 4–16 hours; pressure just as varied	12
<i>Air oven method.</i> —Temperature 100°–110°C.; time 3½–16 hours	10
<i>Water ovens</i> or water and air ovens in varying lengths of time	5
No determination made	7

The laboratories supplied with vacuum ovens were satisfied with the results obtained. Some air-oven users thought the results were close enough to the true value for routine work, but the larger number expressed dissatisfaction and desired a more accurate standard method for those who were unable to secure vacuum ovens.

A method outlined by Coleman¹ is similar to one which has been in use in the associate referee's laboratory for about five years. It is impossible to state where the method originated, but it was passed on by another chemist. It gives good results as compared to the toluene and vacuum oven methods.

This method is outlined as follows:

Approximately 2 grams of material is weighed into a flat shallow metal dish with a tightly fitting cover and placed in an air oven for 1 hour at 130°C. At the expiration of this time the covers are placed on the dishes, and the whole is transferred to a desiccator, cooled to room temperature, and weighed.

The associate referee found that while the method may give concordant results with flour, a temperature of 135°C. and time ranging from 2 to 3 hours are required for other types of feeds.

While it may be argued—and the point well taken—from the figures obtained in the experimental work and from work performed by others² that they do not represent their true value, it may be pointed out that the results are much closer to a vacuum-oven determination than those obtained by drying at 100°–110°C. for any length of time.

EXPERIMENTAL WORK.

For the purpose of this report, work was confined to wheat mill by-products such as are used in Canada: bran with a fiber content of about 10.5 per cent, shorts with 8 per cent, middlings with 4.5 per cent, and feed flour with 2.0 per cent.

¶ Samples of these products were ground, where necessary, to pass through a 1 mm. sieve, thoroughly mixed, and preserved in small tightly stoppered bottles. One set was sent to the chemical laboratory of the

¹ *Cereal Chem.*, 1927, 4: 311.

² *This Journal*, 1925, 8: 354.

Central Experimental Farm with a request that a moisture determination be carried out following strictly the vacuum oven method¹. A second set was sent to the chemical laboratory of the Customs Department with the request that moisture be determined in an air oven at 105°C., according to their method, which is 5 hours. The remaining determinations were made in the associate referee's laboratory.

The results may be tabulated as follows:

Results of moisture determinations showing relative amounts obtained by different methods.

LABORATORY EXPERIMENTAL FARM, DEPARTMENT OF AGRICULTURE, OTTAWA.

Heated for 5 hours at 100°C. in a vacuum of approximately 25 inches.

	BRAN	SHORTS	MIDLINGS	FEED FLOUR
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	14.58	12.95	12.69	13.06
	14.62	12.77	12.72	13.04
	14.63	13.04	12.75	13.16
	14.72	12.94	12.73	13.15
Average	14.64	12.93	12.72	13.10
Variation	0.14	0.27	0.04	0.11

SEED BRANCH CHEMICAL LABORATORY, OTTAWA.

135°C. in an air oven—weighed every hour.

<i>hours</i>				
1	13.70	12.55	11.70	12.30
2	14.75	13.15	12.30	12.80
3	14.95	13.20	12.55	12.90
4	14.80	13.20	12.60	12.90

135°C. in an air oven—weighed at end of second hour.

14.85	13.25	12.55	12.95
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130°C. in an air oven—weighed at end of second hour.

13.80	12.70	12.25	12.80
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Air oven at 105°C.—different weighings.

<i>hours</i>				
3½	13.75	12.35	11.45	11.93
5	13.50	11.85	11.10	11.38
14	13.90	12.43	11.40	11.85

CUSTOMS LABORATORY

105°C. in an air oven for 5 hours.

13.62	12.05	11.19	11.55
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¹ *Methods of Analysis*, A. O. A. C., 1925, 115.

REPORT ON SUGARS AND SUGAR PRODUCTS.

By R. T. BALCH (Bureau of Chemistry and Soils, Washington, D. C.),
*Acting Referee*¹.

The work of this division during the current year is represented by reports from the Associate Referees on Honey, on Polariscopic Methods, and on Chemical Methods for Reducing Sugars. As the Associate Referee on Maple Products was unable to complete his work, no report was submitted. No active work was accomplished by the Associate Referee on Drying, Densimetric and Refractometric Methods, and the Refereeship for Starch Conversion Products is still unfilled.

With regard to the reports submitted, the acting referee wishes to make the following comments:

The results of this year's work on polariscopic methods indicate more conclusively the value of the invertase method for sucrose determination and the unsuitability of the acid inversion methods for other than the analysis of pure sugar mixtures. The recommendations by this associate referee appear to be well founded and are approved.

The detection of added invert sugar in honey has been a subject of considerable investigation. Schuette has recommended collaborative study of Auerbach and Bodländer's reduction method of determining glucose and obtaining fructose by difference from total reducing sugars. It is believed that there are other dependable ways of analyzing honeys for their fructose content, namely, polariscopically at elevated temperatures and by Jackson's modification of Nyns' method of selective determination of fructose, which should be included in the recommendations for study during the ensuing year.

As the Associate Referee on Chemical Methods for Reducing Sugars will point out, there is considerable need for revision of these methods of sugar analysis. In addition to the recommendations² submitted, in which the referee concurs, it would also be desirable to direct attention to a study of methods for determining very small quantities of reducing sugars in the presence of large quantities of disaccharides.

From this brief report, it may be noted that an extensive program is outlined for the coming year, and it is hoped that more active work may be done in the remaining sub-divisions along lines previously recommended.

¹ In place of H. S. Paine, resigned.

² For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11. 66.

REPORT ON HONEY.

By H. A. SCHUETTE¹ (Department of Chemistry, University of Wisconsin, Madison, Wis.), *Associate Referee*.

The possibility of making invert sugar from sucrose by enzyme conversion lends strength to the thought that the former variety rather than that produced by acid hydrolysis might be used in the preparation of sophisticated honeys and so escape detection if those tests for its presence be used, which, like that of Fiehe², depend upon the qualitative identification of a by-product of the inversion.

A comparatively recent method for distinguishing between true and adulterated honeys, which rests upon no such premise as the foregoing, is that proposed by Auerbach and Bodländer³. The essential feature of this method is the establishment of the relative quantities of glucose and fructose present. Since fructose, as a rule, predominates over glucose in genuine natural honey, while invert sugar itself consists of an equal mixture of these sugars, this ratio should be greater than unity in genuine honeys and should fall below it when invert sugar has been substituted wholly or in part for the natural variety.

The basic principle of the Auerbach-Bodländer method rests upon a fundamental reaction of formaldehyde, announced some years ago by Romijn⁴, by virtue of which it is readily oxidized by iodine in the presence of alkalis. Romijn then expanded his method to include the determination of aldose sugars⁵. Later Willstätter and Schudel⁶, Behre⁷, Kolthoff⁸ and others successfully applied this method, and finally the authors in question adapted it to the analysis of honey⁹.

Their method of procedure follows:

The sample of honey is heated in a closed container, at a temperature not above 50°C. Heating should be continued until a clear solution is obtained, or at least until a homogeneous mixture is formed.

Samples weighing 2 grams are washed with water into a 250 cc. volumetric flask, and when solution is complete, the volume is completed to the mark with water. Of this solution 25 cc. portions are taken for the determination of the total reducing sugars and the glucose, respectively. The determination of the former is made gravimetrically; that of the latter, iodometrically.

For the determination of the glucose content, there is added to 25 cc. of the dilute honey solution such an amount of a 0.1 *N* iodine solution that at least one-half or one-third of the latter is in excess. This is followed by the addition of 100 cc. of a mixture consisting of equal volumes of 0.2 *M* sodium bicarbonate and sodium carbonate solu-

¹ Presented by R. T. Balch.

² *Z. Nahr. Genussm.*, 1908, 16: 75.

³ *Ibid.*, 1924, 47: 233.

⁴ *Z. anal. Chem.*, 1897, 36: 18.

⁵ *Ibid.*, 349.

⁶ *Ber.*, 1918, 51: 780.

⁷ *Z. Nahr. Genussm.*, 1921, 41: 226; 42: 242.

⁸ *Ibid.*, 1923, 45: 131.

⁹ *Z. angew. Chem.*, 1923, 36: 602.

tions. The whole is then allowed to stand in the dark for $1\frac{1}{2}$ -2 hours, at the end of which time it is acidified by the addition of 12 cc. of 25 per cent sulfuric acid solution and the excess of iodine is determined in the usual manner with 0.1 *N* sodium thiosulfate solution. A blank determination is made on the reagents concurrently with the determination of the glucose.

The difference between the amount of 0.1 *N* thiosulfate solution required by the blank and that necessary to reduce the excess of iodine, multiplied by 0.009005, represents the glucose content of the sample under analysis. The average of the glucose content found by at least two parallel determinations is subtracted from the average value found for total reducing sugars. The difference is expressed as fructose. Its relation to the glucose content is then calculated.

The authors of this method have apparently anticipated certain criticisms of their procedure for they state that this difference does not represent the exact fructose content because of the reducing action of certain constituents of honey other than glucose and fructose, which, however, are present in small quantity. They emphasize the fact that, for all practical purposes, interest centers upon the difference between reducing sugars and glucose, and that this difference represents the order of magnitude of the fructose content of the honey under analysis when the determination of the latter is made by a prescribed iodometric procedure.

Aldoses other than glucose are susceptible to oxidation by iodine, but inasmuch as they are not met with in genuine or sophisticated honeys consideration need not be given to this fact.

As a result of the examination of a number of German honeys, during the course of which the effect of such variables as age and temperature of heating the samples were studied, the authors set the minimum limiting glucose-fructose ratio for genuine honey at 100 : 106. Gronover and Wohnlich¹, however, are of the opinion that this ratio is too high.

Preliminary studies were made in this laboratory of the proposal of Auerbach and Bodländer to differentiate between genuine and adulterated honeys by utilizing the differences which obtain in their relative quantities of glucose and fructose. Attention was centered upon the time factor as influencing the oxidation of the glucose, a study of the technic of the determination itself, and the effect upon the glucose-fructose ratio of several honeys progressively adulterated with a commercial invert sugar "mush" and an invert sugar preparation used by manufacturers of confectionery.

The time factor in the oxidation of glucose to gluconic acid under the conditions of this reaction is one that apparently must be rigidly adhered to if concordant and reproducible results are to be obtained. The original recommendations are in substance that the action of the iodine upon the glucose is to be stopped at the end of $1\frac{1}{2}$ -2 hours. It was found, however, that equilibrium was not reached with the experimental

¹ *Z. Nahr. Genussm.*, 1924, 48: 405.

honeys used for some 3 to 4 hours and that, therefore, a very clearly defined reaction period must be established in the interest of uniformity of results. This period may well be set at 2 hours. The data in Table 1 are pertinent to the subject.

Although the number of samples of pure honey examined was not sufficient to yield data in final confirmation or disproval at this time of the validity of applying Auerbach and Bodländer's 1 : 1.06 ratio to American grown honeys, yet it would seem that this ratio possesses merit (Table 2). In every instance the presence of added invert sugar was apparent.

Grateful acknowledgment is made to H. Isabel Dow for the invaluable aid given in making possible this report. Miss Dow carried out the analytical work, part of which is herein embodied.

RECOMMENDATIONS.

It is recommended that the procedure of Auerbach and Bodländer for the detection of adulteration of honey with commercial invert sugar be the subject of collaborative study.

TABLE 1.

Effect of time upon the iodometric determination of glucose in honey.

TIME	GLUCOSE	FRUCTOSE BY DIFFERENCE	GLUCOSE- FRUCTOSE RATIO
hours	per cent	per cent	
1.0	34.04	39.73	1 : 1.17
1.5	34.06	39.71	1 : 1.17
2.0	34.45	39.32	1 : 1.14
3.0	35.44	38.34	1 : 1.08
4.0	35.34	38.43	1 : 1.09

TABLE 2.

Analysis of a honey progressively adulterated with commercial invert sugar.

INVERT SUGAR ADDED	REDUCING SUGARS AS INVERT	GLUCOSE	FRUCTOSE BY DIFFERENCE	GLUCOSE- FRUCTOSE RATIO
per cent	per cent	per cent	per cent	
SERIES "A"				
0.0	75.04	34.79	40.25	1 : 1.16
9.7	74.78	37.58	37.20	1 : 0.99
50.6	76.97	39.86	37.11	1 : 0.93
100.0	74.42	39.25	35.17	1 : 0.89
SERIES "B"				
none	75.04	34.79	40.25	1 : 1.16
1.6	74.37	36.88	37.49	1 : 1.02
10.0	74.59	37.78	36.81	1 : 0.97
100.0	74.42	39.25	35.17	1 : 0.89

No report on maple products was given by the associate referee.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 66.

No report on starch conversion products was given, as no associate referee was appointed.

No report on drying, densimetric, and refractometric methods was given by the associate referee.

REPORT ON POLARISCOPIC METHODS.

By F. W. ZERBAN¹ (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*.

The associate referee's report for 1925² contained the following recommendations for further work:

(4) That the investigation presented under A (Determination of sucrose in the absence of raffinose) be continued, sucrose, invert sugar, reversion products, and also other non-sugars occurring in saccharine products not derived from the beet, being used.

(5) That the investigation presented under B (Determination of sucrose and raffinose by the two-enzyme method of Paine and Balch) be repeated next year, the quantities of raffinose used being extended beyond this year's limit of 3 per cent.

Lack of time prevented carrying out recommendation (5). The work recommended under (4) was started last year by preparing a sirup that contained the non-sugars specified. The method used to make this sirup from cane blackstrap is described in detail in the associate referee's report for 1926³. An analysis of the sirup gave total solids 48.90 per cent, sucrose 17.17 per cent, reducing sugars 19.38 per cent, ash 6.72 per cent, and organic non-sugars 5.63 per cent. The color was light enough to permit Clerget determinations without further clarification.

Finally, the sucrose contained in this sirup was inverted by means of commercial invertase, the mixture being allowed to stand for several months at room temperature. In spite of this precaution the sirup darkened considerably. When inversion was practically complete, a small quantity of Suchar was added, and the mixture was heated to 80°C., quickly cooled, and filtered under suction. This treatment destroyed the invertase, and at the same time redecolorized the sirup. A part of this final product was sent to the Carbohydrate Laboratory of the Bureau of Chemistry and Soils, with the following directions for the analytical work:

Make the analyses by following the directions as given in 1924⁴. Use a sirup prepared by decolorizing cane blackstrap, inverting the sucrose contained in it by means of invertase, and adding known quantities of sucrose.

¹ Presented by R. T. Balch.

² *This Journal*, 1926, 9: 166.

³ *Ibid.*, 1927, 10: 183.

⁴ *Ibid.*, 1925, 8: 384.

Use the following concentrations, without lead clarification, for both the direct and the invert polarization:

- (1) Sucrose (Domino tablet sugar), 13 grams in 100 cc.
- (2) Sirup alone, 27.54 grams (=13 grams solids) in 100 cc.
- (3) Mixture of 20.65 grams (=9.75 grams solids) sirup and 3.25 grams sucrose in 100 cc.
- (4) Mixture of 13.77 grams (=6.5 grams solids) sirup and 6.5 grams sucrose in 100 cc.
- (5) Mixture of 6.88 grams (=3.25 grams solids) sirup and 9.75 grams sucrose in 100 cc.

Use the following methods of analysis:

- (a) Official invertase method at room temperature, *Methods of Analysis, A. O. A. C.*, 1925, p. 185, 22 (a) and (b), with one modification: use 10 cc. invertase of $k = 0.1$.
- (b) Official acid method at room temperature, *Methods of Analysis, A. O. A. C.*, 1925, p. 187, 23 (c).
- (c) Jackson and Gillis method No. II (U. S. Bur. Standards Scientific Paper 375, p. 182 (g), and pp. 184-185).
- (d) Jackson and Gillis method No. IV (U. S. Bur. Standards Scientific Paper 375, p. 182 (g), and pp. 187-188).

Make the solutions to be used for the direct reading nearly to the mark and allow to stand overnight to complete mutarotation.

Also allow the solutions to be used for the invert reading to stand overnight, at about 26°-27°C.

Make the readings at 20°C. in a water-jacketed tube.

All the determinations were run in duplicate—in Washington by M. A. McCalip, and in this laboratory by C. A. Gamble and J. E. Mull, who made one each. The writer wishes to express his thanks to these collaborators. Owing to a misinterpretation of the directions, McCalip used concentrations that were only one-half of those given in the directions; furthermore, he made the inversions according to Jackson and Gillis methods No. II and IV by heating to 50°C. for 29 minutes, not at room temperature, as specified.

The results of all the analyses are shown in the tables, which are arranged like those given in the report for 1925. The following Clerget divisors were used in the calculations:

(1) Official invertase method: $132.1 + 0.073 (m - 13)$. It will be noted that the new concentration factor, 0.073, recently established by Paine and Balch¹ has been substituted for the old factor 0.0676. The latter, however, has been retained in the other methods.

- (2) Official acid method: $133.2 + 0.0676 (m - 13)$.
- (3) Jackson and Gillis method No. II: $133.34 + 0.0676 (m - 13)$.
- (4) Jackson and Gillis method No. IV: $132.63 + 0.0676 (m - 13)$.

In all the formulas given, m denotes the number of grams of total solids (not sucrose) in 100 cc. This point is emphasized because previous reports by the associate referee have definitely proved that the water

¹ *J. Am. Chem. Soc.*, 1927, 49: 1019.

concentration, not the sucrose concentration, regulates the factor in the Clerget divisor. As in former reports, the percentage figures for the sucrose are given on the basis of 26 grams in 100 cc. For the sirup and for the mixtures the results are expressed as sucrose in percentage of total solids.

TABLE 1.

Sucrose.

ANALYST, AND METHOD USED	DIRECT POLARIZATION	INVERT POLARIZATION	PERCENTAGE
Invertase method			
M. A. McCalip	24.95	— 7 895	24 95
C. A. Gamble, J. E. Mull	49.90	—16 08	49.95
A. O. A. C. acid method			
M. A. McCalip	24 95	— 8 115	24 91
C. A. Gamble, J. E. Mull	49.90	—16.60	49.93
Jackson and Gillis method No. II			
M. A. McCalip	24.80	— 8 33	24 93
C. A. Gamble, J. E. Mull	49.60	—16.98	49.93
Jackson and Gillis method No. IV			
M. A. McCalip	24 80	— 8 125	24 91
C. A. Gamble, J. E. Mull	49.68	—16 60	49.97

Sirup prepared from blackstrap by decolorization and inversion.

Invertase method			
M. A. McCalip	— 6 55	— 6.595	0 14
C. A. Gamble, J. E. Mull	—13 33	—13.48	0 23
			0.19
A. O. A. C. acid method			
M. A. McCalip	— 6 55	— 6 62	0.21
C. A. Gamble, J. E. Mull	—13.33	—13.48	0 23
			0 22
Jackson and Gillis method No. II			
M. A. McCalip	— 6.815	— 6 886	0 21
C. A. Gamble, J. E. Mull	—13.85	—14.10	0 37
			0.29
Jackson and Gillis method No. IV			
M. A. McCalip	— 6 735	— 6 615	—0.36
C. A. Gamble, J. E. Mull	—13.75	—13.48	—0 41
			—0 39

As shown in the tables, the agreement between the results found and those calculated is not quite so good as had been noted in previous work. This may be ascribed to two causes. In the first place the sirup, which was very light when freshly decolorized, darkened rather rapidly on standing, even in the refrigerator. Although clarification could be dispensed with, the solutions at the time of analysis were not nearly so light in color as those analyzed in 1924 and in 1925. They were, therefore, not so easy to read, particularly because in colored solutions the discrepancy between the rotation dispersion of quartz and of invert sugar is very disturbing when large quantities of invert sugar are present

TABLE 2.
Mixture I—one part sucrose and three parts sirup solids.

ANALYST, AND METHOD USED	DIRECT POLARIZA- TION	INVERT POLARIZA- TION	SUCROSE FOUND per cent	SUCROSE CALCULATED ON BASIS OF SAME METHOD per cent
Invertase method				
M. A. McCalip	1.31	— 6.995	25.24	
C. A. Gamble, J. E. Mull	2.45	—14.13	25.10	
			25.17	25.11
A. O. A. C. acid method				
M. A. McCalip	1.31	— 7.025	25.11	
C. A. Gamble, J. E. Mull	2.45	—14.18	24.97	
			25.04	25.10
Jackson and Gillis method No. II				
M. A. McCalip	1.035	— 7.31	25.12	
C. A. Gamble, J. E. Mull	2.00	—14.70	25.05	
			25.09	25.17
Jackson and Gillis method No. IV				
M. A. McCalip	1.115	— 7.025	24.63	
C. A. Gamble, J. E. Mull	2.20	—14.18	24.70	
			24.67	24.66

Mixture II—equal parts of sucrose and sirup solids.

Invertase method				
M. A. McCalip	9.15	— 7.305	50.00	
C. A. Gamble, J. E. Mull	18.20	—14.78	49.93	
			49.97	50.02
A. O. A. C. acid method				
M. A. McCalip	9.15	— 7.41	49.89	
C. A. Gamble, J. E. Mull	18.20	—15.03	49.90	
			49.90	49.99
Jackson and Gillis method No. II				
M. A. McCalip	8.945	— 7.665	49.99	
C. A. Gamble, J. E. Mull	17.73	—15.53	49.89	
			49.94	50.04
Jackson and Gillis method No. IV				
M. A. McCalip	9.015	— 7.41	49.70	
C. A. Gamble, J. E. Mull	17.90	—15.03	49.66	
			49.68	49.70

Mixture III—three parts sucrose and one part sirup solids.

Invertase method				
M. A. McCalip	17.065	— 7.625	75.02	
C. A. Gamble, J. E. Mull	34.00	—15.53	74.99	
			75.01	74.94
A. O. A. C. acid method				
M. A. McCalip	17.065	— 7.81	74.95	
C. A. Gamble, J. E. Mull	34.00	—15.83	74.82	
			74.89	74.87
Jackson and Gillis method No. II				
M. A. McCalip	16.91	— 8.03	75.07	
C. A. Gamble, J. E. Mull	33.70	—16.23	74.89	
			74.98	74.92
Jackson and Gillis method No. IV				
M. A. McCalip	16.93	— 7.81	74.86	
C. A. Gamble, J. E. Mull	33.80	—15.83	74.84	
			74.85	74.75

and the ordinary bichromate cell is used. In the second place, the errors in the readings obtained in one of the laboratories with a concentration of 6.5 grams of solids in 100 cc. are multiplied by four. Under these circumstances, the maximum difference of 0.1 per cent between the average results found and those calculated for the percentage of sucrose in the mixtures does not appear to be excessive.

As was the case two years ago, the sucrose used in one of the laboratories was of a slightly lower polarization than that employed in the other. However, the average results are strictly comparable since the same weights of sucrose and sirup were used in both laboratories.

The results of the sirup analyses at first sight were very surprising because it was recalled that in the 1925 report an invert sugar sirup containing no reversion products, and only 0.08 per cent uninverted sucrose, showed an apparent sucrose content of 0.73 per cent by the plain acid method. The two Jackson and Gillis methods, however, gave results agreeing with those obtained by the invertase method. When reversion products were present in addition to invert sugar, the two Jackson and Gillis methods revealed them as apparent sucrose, but even then gave results lower than the plain acid method, because the effect of the free acid on the levulose is eliminated by neutralization of the acid, or by adding an equivalent quantity of sodium chloride to the solution used for the direct reading. When these previous results are considered, it would have been expected that with the invert sirup prepared from blackstrap the plain acid method would produce results showing a high apparent sucrose content, and that the two Jackson and Gillis methods would produce a figure somewhere between that obtained by the plain acid method and that obtained by the invertase method.

However, the actual results were entirely different from what had been expected. The plain acid method gave practically the same results as the invertase method. The results obtained by Jackson and Gillis method No. II were also the same as, or a trifle higher than, those obtained by the first two methods. But Jackson and Gillis method No. IV gave consistently an invert reading lower than the direct reading, which indicated apparent *negative* sucrose.

Provided hydrolysis by the acid used was really complete in all cases, these results may be explained on the basis of the following considerations. It has long been known that cane-sugar products, like beet-sugar products, contain optically active non-sugars which change their rotation with acidity. Without going into the entire literature on this subject, it may be mentioned that Browne¹ studied the distribution of the various forms of nitrogen in a cane blackstrap and found that of the 0.467 per cent of total nitrogen, 38.00 per cent was in the form of amino acids and 14.38

¹ Louisiana Agr. Expt. Sta. Bull. 91, p. 93.

per cent in the form of amids of amino acids. This percentage would correspond roughly to a total of 2 per cent of asparagine and aspartic acid, calculated on the molasses itself. Some years later the writer¹ isolated l-asparagine together with small quantities of d-glutamine and of tyrosine from sugar cane juice. These substances are not removed by the ordinary methods of clarification and consequently they accumulate in the molasses. The prolonged heating, however, hydrolyzes a part of the amids, converting them into the corresponding amino acids. For this reason molasses contains more of the amino acids, while in cane juice the amids predominate. Employing Ambler's² modification of the ninhydrin method, Gamble made a determination of amino nitrogen in the sirup used in this year's investigation and found 0.13 per cent, which would be equivalent to 1.2 per cent aspartic acid or 1.4 per cent asparagine.

The optical properties of these substances are as follows³: l-Asparagine is slightly levorotatory, $\left[\alpha\right] \frac{20}{D} = -5.51$ to 7.95 , depending on the concentration. Addition of acid reduces the levorotation and then produces dextrorotation, which increases with the hydrogen-ion concentration to $\left[\alpha\right] \frac{20}{D} = +34.3$. l-Aspartic acid shows $\left[\alpha\right] \frac{20}{D} = +4.36$; in the presence of acid it is also dextrorotatory, the numerical values being about the same as for asparagine. d-Glutamine is dextrorotatory, $\left[\alpha\right] \frac{20}{D} = +6.94$ to 10.24 , depending on the concentration. In the presence of acid the dextrorotation increases and reaches about the same value as that of asparagine in acid solution. d-Glutaminic acid shows dextrorotation of about the same order as d-glutamine, under the same conditions of acidity. Tyrosine, of which only a trace was found in cane juice, has $\left[\alpha\right] \frac{20}{D} =$ about -8 , in acid solution. Clarification with lead subacetate does not remove the amino acids and amids; on the contrary with some of them it produces even greater changes in optical rotation than free acid does. However, no further discussion of this point is pertinent here, because in the analyses reported no clarifying agent was used.

Since cane blackstrap probably contains mostly l-asparagine and l-aspartic acid, with some d-glutamine and d-glutaminic acid, it might be expected that this mixture would be only slightly optically active, either positive or negative, in water solution, while in the presence of the free acid used for inversion the mixture would become strongly dextrorotatory. The same should be true of the sirup prepared from blackstrap.

¹ Orig. Com. 8th. Intern. Congr. Appl. Chem., 8: 103.

² Intern. Sugar J., 1927, 29: 382.

³ Eisenschimmel, Z. Zuckerind. czechoslovak. Rep., 1927, 51: 337; Landolt. Das Optische Drehungsvermögen, 2nd ed., 1898, 476, 486-488.

These considerations, in connection with the facts related in previous reports, offer at least a general explanation of the unexpected results obtained with the sirup analyzed this year. When Jackson and Gillis method No. IV is used, the free acid present in the inverted solution causes strong dextrorotation in the amids and amino acids, and this action more than compensates for the levorotation caused by the hydrolysis of the reversion products present. So far it is not known just what effect is exerted by the sodium chloride added to the solution for the direct polarization, but it evidently does not compensate for the change in rotation of the amids and amino acids. This fact has been recognized by Jackson and Gillis, but they recommended their method No. IV for the analysis of cane products, assuming that they are free from these nitrogenous substances.

When the plain acid method is used, the strong dextrorotation of the amids and amino acids, due to the effect of the free hydrochloric acid, is counteracted by the levorotation of the hydrolyzed reversion products, plus the increase in the levorotation of fructose in acid solution. In the particular sirup investigated this year, the two effects just about balance each other, but in products of different composition the plain acid method may conceivably give either higher or lower results than the invertase method.

Even with Jackson and Gillis method No. II, the rotation of the amid-amino-acid mixture after inversion and neutralization is probably different from that before inversion. Any amids present would be largely hydrolyzed upon heating or standing in the presence of acid. The change of levorotatory asparagine into dextrorotatory aspartic acid would tend to decrease the levorotation of the inverted solution, and in the absence of reversion products would indicate negative sucrose. Therefore, the relative quantities of amids and amino acids, and of reversion products, will still be the deciding factors as to whether the sucrose result will be greater or smaller than when obtained by invertase inversion. In the particular sirup under investigation the two opposite effects nearly balanced each other.

What has been said in regard to the sirup itself, applies equally to the three mixtures of sucrose and sirup. Each method yields correct results, within the limits of error, when the calculations are based on the sucrose and sirup results obtained by the same method. But when the comparisons are made with the invertase method, Jackson and Gillis method No. IV gives too low results with all three mixtures, while the plain acid method and Jackson and Gillis method No. II, through a compensation of errors, yield practically the same results as the invertase method, especially in the mixtures containing a large proportion of sucrose.

The investigation carried out this year, considered in connection with the previous reports, shows that in the analysis of a cane-sugar product

containing sucrose, invert sugar, reversion products, and optically active amids and amino acids, the following deductions may be made:

(1) The results obtained by the plain acid method are affected by the influence of the free acid on the rotation of the levulose, the tendency being towards high results; by the hydrolysis of the reversion products, the tendency also being towards high results; and by the changes in rotation caused by the effect of the acid on the amid-amino-acid mixture, the tendency being towards low results.

(2) The results obtained by Jackson and Gillis method No. II are not affected by the influence of the free acid on the rotation of the levulose, but they are affected by the hydrolysis of the reversion products, the tendency being towards high results, and by the hydrolysis of any asparagine present, the tendency being towards low results.

(3) The results obtained by Jackson and Gillis method No. IV are not affected by the influence of the free acid on the rotation of the levulose, but they are affected by the hydrolysis of the reversion products, the tendency being towards high results, and by the change in rotation of the amids and amino acids caused by free acid, the tendency being towards low results. This last-named effect is greater than that caused by the hydrolysis of asparagine followed by neutralization, mentioned under (2), and for this reason the results obtained by Jackson and Gillis method No. IV are, for the same product, lower than by method No. II.

Any of the three methods in which acid is used for hydrolysis may give results either higher or lower than the true value, or, by a compensation of the various errors, a correct result. When the percentage of sucrose in a product is high, and that of the mixture of invert sugar, reversion products, amids, and amino acids is low, the discrepancies caused by the latter substances may fall within the limits of error of saccharimetric analysis.

Summarizing, it may be stated that with complex mixtures, like many of the products derived from the sugar cane, the only safe procedure for the determination of sucrose is the invertase method, although in special cases the two Jackson and Gillis methods, or even the plain acid method may, by a compensation of errors or by a negligible magnitude of the errors, give correct results.

RECOMMENDATIONS¹.

In view of the fact that the explanation for the results obtained this year is largely based on previous literature, which did not take the application to the analysis of cane products into consideration, it is recommended—

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 67.

(1) That a study be made of the four inversion methods used in this investigation, as applied to mixtures of pure sugars and pure amids and amino acids.

(2) That the isolation in the pure state of the amids and amino acids occurring in cane blackstrap be attempted.

The sirup used in this year's investigation offers a good opportunity to study the effect of lead clarification, combined with deleading, on the results of the Clerget method. It is therefore also recommended—

(3) That the sirup in which sucrose determinations have been made by various modifications of the Clerget method without previous clarification be used also for Clerget determinations preceded by lead clarification and deleading.

REPORT ON CHEMICAL METHODS FOR REDUCING SUGARS.

By R. F. JACKSON (Bureau of Standards, Washington, D. C.),
Associate Referee.

In a previous report the associate referee has called attention to the duplication of analytical processes in the association's methods of analysis for reducing sugar. This duplication not only occupies valuable space in the form of voluminous tables, but it is a source of confusion to the analyst. Thus, for example, there are duplicate methods for invert sugar, for dextrose, for lactose, and for maltose, differing only in slight details of procedure, but each modification requires a full tabulation of the copper and sugar values.

It is obvious that the selection between duplicate methods should be made with deliberation. Consequently, the associate referee desires at this time to suggest a selection between but one pair of duplicate methods, namely those for invert sugar. The present official methods for the determination of invert sugar¹ are that of Munson and Walker and that of Meissl. Both methods specify 50 cc. of mixed Soxhlet reagent, 50 cc. of sugar solution, a preliminary heating of 4 minutes, and a time of boiling of 2 minutes. They differ solely in but one specification. Munson and Walker prescribe an immediate filtration at the expiration of 2 minutes, while Meissl prescribes the addition of 100 cc. of cold, recently boiled water before filtration. The tables accompanying these respective methods differ by about 2 per cent. From an *a priori* viewpoint, the method of stopping the reaction by adding cold water is of very doubtful efficacy. It is a matter of considerable difficulty to prepare cold water free from air, and the act of pouring a large volume of water inevitably carries a quantity of air in finely divided state. Thus, the back oxidation

¹ *Methods of Analysis*, A. O. A. C., 1925, 189.

of copper may very easily cause errors as great as the slightly greater uncertainty of time of the reaction. Moreover, the uncertainty in the time of boiling is at the beginning of the period rather than at the end. The immediate filtration of the boiling solution at the end of the reaction prescribed by Munson and Walker requires but 10–15 seconds and is carried out in analysis in exactly the same manner as in standardization. Consequently, a variation of only a few seconds is possible between different analyses.

In a survey of modern literature the associate referee has discovered no reference to Meissl's method; it is apparent then that in its practical application it has become obsolete. The ostensible reason for its retention thus far is its possible application to Meissl and Hiller's method when a solution upon complete analysis has been found to have contained no sucrose. Meissl and Hiller's table, however, contains a column which provides for this case and so makes Meissl's table for invert sugar alone superfluous.

The associate referee, therefore, recommends that Meissl's method for invert sugar be discarded and that the first three lines on page 195, *Methods of Analysis*, and Table 10, pp. 448–449, be deleted.

The gravimetric methods in the present list are applicable to analyses of solutions containing from 2 to 4 mg. to 250 mg. of dextrose—or 340 mg. of the disaccharides—in 50 cc. The lower ranges of concentration, however, are involved in greater uncertainty than the ranges above concentrations of 50 mg. Auto-reductions of the copper reagents and small changes in the weight of asbestos may become very large percentages of the reduced copper. There are, on the other hand, methods that can be used to the best advantage with concentrations of sugar for which the gravimetric tables are least reliable. Many of these methods are volumetric and thus rapid and convenient. Among those which have been considered are Benedict's method¹, which is particularly adaptable to urine analysis, and the iodometric method of Scales².

W. D. Chase of the Bureau of Standards has experimented extensively with these methods. He has found that Benedict's method is highly reliable under rigorously specified conditions but that there appears to be some doubt in regard to its general applicability. In view of this uncertainty the associate referee recommends that it be subjected to further study. The method of Scales in various modifications has been the subject of four published investigations by three authors³, in each of which it has been found satisfactory.

Chase, the associate referee, and other analysts at the Bureau of Standards have found it as satisfactory as claimed by the author. It satisfies

¹ *J. Biol. Chem.*, 1909, 5: 485.

² *J. Ind. Eng. Chem.*, 1919, 11: 747.

³ *Cambridge Lancet*, 1917, 192: 613; *Clark. J. Am. Chem. Soc.*, 1918, 40: 1759; *Scales. J. Ind. Eng. Chem.*, 1919, 11: 747; *J. Biol. Chem.*, 1915, 23: 81.

to a high degree the need of a volumetric method at low concentrations of sugar and overlaps the lower ranges of concentration of the volumetric method of Lane and Eynon¹. For a considerable range of concentration the ratio of titer to concentration of sugar is constant and thus the need of empirical tables is avoided. Its useful range of concentration lies between 1.5–20 mg. of sugar in 10 cc. It compels the analyst to standardize his own thiosulfate solution and thus eliminates to a great extent the personal equation. In view of these advantages the associate referee recommends that the volumetric method of Scales be accepted as a tentative method of this association. The method is as follows:

SCALES METHOD.

REAGENTS.

(a) *Benedict's solutions*.—Dissolve 16 grams of copper sulfate in 125–150 cc. of water. Then dissolve 150 grams of sodium citrate, 130 grams of sodium carbonate (anhydrous), and 10 grams of sodium bicarbonate in about 650 cc. of water, heating to accelerate solution. Combine the two solutions, with stirring. Cool, make to 1 liter, and filter.

(b) *Iodine solution*.—0.04 *N*.

(c) *Thiosulfate solution*.—0.04 *N*.

(d) *Hydrochloric acid solution*.—60 cc. of concentrated acid per liter.

(e) *Acetic acid solution*.—24 cc. of glacial acid per liter.

(f) *Soluble starch solution*.

PROCEDURE.

Transfer 20 cc. of copper reagent to a 300 cc. Erlenmeyer flask fitted with a 2-hole rubber stopper. Add 10 cc. of sugar solution containing less than 20 mg. of reducing sugar. Place over a flame, bring to boiling in 4 minutes, and continue the boiling for exactly 3 minutes. (If preferred, an electric hot plate may be used, in which case a period of 5 minutes is required to raise the solution to the boiling point.) At the expiration of 3 minutes from the beginning of the boiling, cool rapidly by holding under a cold water faucet, add 100 cc. of dilute acetic acid from a graduate, and transfer an exactly measured 0.04 *N* iodine solution. Add 25 cc. of dilute hydrochloric acid from a pipet held against the side of the flask, and agitate to distribute the acid rapidly. Rotate the flask for 1 minute to insure the solution of all cuprous chloride. Titrate excess iodine with thiosulfate solution.

For amounts less than 20 mg. of sugar each cc. of thiosulfate will represent a constant quantity of sugar; for dextrose, approximately 1.11 mg. per cc.

The associate referee suggests the need of a method for the estimation of minute quantities of reducing sugar. Such methods have long been in use in the analysis of blood and other body fluids. It is perhaps obvious that its extension into the field of agricultural chemistry would serve a useful purpose. Chase and the writer have, for example, employed it in the investigation of the early stages of hydrolysis of the polysaccharides in the artichoke with satisfactory results. The method is capable of estimating 0.1 mg. of sugar with a precision of about 1 per cent. The associate referee recommends that Benedict's modification² of the Folin and Wu³ method be accepted as a tentative method.

¹ *J. Soc. Chem. Ind.*, 1923, 42: 32.

² *J. Biol. Chem.*, 1926, 68: 759.

³ *Ibid.*, 1919, 38: 81; 1920, 41: 367.

BENEDICT'S MODIFICATION OF THE FOLIN AND WU METHOD.

REAGENTS.

(a) *Alkaline copper solution*.—Dissolve 200 grams of sodium citrate and 60 grams of sodium carbonate together in about 800 cc. of water. Then dissolve 6.5 grams of pure copper sulfate crystals separately in about 100 cc. of water and add to the former solution with agitation. Add 9 grams of ammonium chloride, dilute to 1 liter, and mix.

(b) *Tungstic acid color*.—Dissolve 100 grams of pure sodium tungstate in about 600 cc. of water in a liter flask. Add 50 grams of pure arsenic pentoxide, then 25 cc. of 85 per cent phosphoric acid and 20 cc. of concentrated hydrochloric acid. Boil 20 minutes. After cooling, add 60 cc. of commercial formalin, 45 cc. of concentrated hydrochloric acid, and 40 grams of sodium chloride. Dilute to 1 liter and mix.

(c) To 100 cc. of the alkaline copper solution add 2.5–3.0 grams of pure anhydrous sodium sulfite and preserve for use not over 1 month.

PROCEDURE.

Transfer 2 cc. of the sugar solution and 2 cc. of the copper reagent to a Folin and Wu sugar tube. Into another tube transfer 2 cc. of a standard dextrose solution containing 0.1 (or 0.2) mg. per cc. and 2 cc. of copper reagent. Mix by side-to-side shaking and place the two tubes in boiling water for 5 minutes. Cool by immersion in cold water and add to each 2 cc. of tungstic acid color reagent. After 1 to 2 minutes dilute to 25 cc., mix thoroughly, and compare in a colorimeter. The sugar in the unknown solution is calculated by the formula—

$$\frac{\text{Depth of column of Standard}}{\text{Depth of column of Unknown}} \times 10 \text{ (or 20)} = \text{milligrams per 100 cc.}$$

RECOMMENDATIONS FOR FURTHER STUDY¹.

In a previous report the associate referee indicated that the method of Nyns² for levulose in the presence of other sugars had proved reliable. In brief, the selective determination of levulose depends upon the fact that at about 49°C. it reduces a copper carbonate solution relatively rapidly, while other sugars are either inactive or reduce it slowly. In the original announcement Nyns states that the reagent is unaffected by glucose. The associate referee finds that this statement is not quite true. If, however, the glucose content is known, the copper recovered can readily be corrected for the glucose reduction and related directly to the levulose in the sample taken. The determination consequently must be made conjointly with a determination of total reducing sugar.

It is recommended that this method be subjected to study during the coming year.

There still remain duplicate methods in the present edition of *Methods of Analysis* for lactose and maltose which differ solely in specifications for time of boiling. It is recommended that these methods be studied for the purpose of selecting the more suitable ones and discarding the others.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 67.

² *Bull. assoc. école sup. brasserie Louvain*, 1925, 25: 63; *C. A.*, 1925, 19: 1236.

COMMITTEES NAMED BY THE PRESIDENT.

Committee to Wait upon Assistant Secretary of Agriculture: C. H. Jones and A. G. McCall.

Committee to Wait upon Honorary President: B. B. Ross and R. N. Brackett.

Committee on Resolutions: W. W. Randall, C. A. Browne, and H. C. Lythgoe.

Auditing Committee: J. W. Sample, J. J. Skinner, and J. D. Weems.

Committee on Nominations: C. H. Jones, R. N. Brackett, H. H. Hanson.

FIRST DAY.

MONDAY—AFTERNOON SESSION.

REPORT ON FERTILIZERS.

By G. S. FRAPS (Agricultural Experiment Station, College Station, Tex.),
Referee.

Considerable progress was made this year. The Associate Referee on Phosphoric Acid completed a long and thorough study of the method of precipitation in the gravimetric method for phosphoric acid and found the official method to be equally as accurate as the other methods tested. He is recommending some minor changes in the official method.

Some workers have claimed that the volumetric method for phosphoric acid gives high results owing to the sulfates present. C. M. Bible¹ of the Mellon Institute states that this objection can be overcome equally as well by modification of the method of precipitation as by precipitation of the sulfates with barium chloride, as recommended by Breckenridge. In view of the fact that the volumetric method is probably used more extensively in control work than is the gravimetric method, it would seem desirable that the volumetric method be reviewed and revised if it is found to be necessary.

The Associate Referee on Nitrogen completed two years' work on methods for nitrate nitrogen in fertilizers. The results fully justify his recommendation that the Jones method be adopted as tentative and his other recommendations. The adoption of the Jones method is particularly desirable in view of the fact that the association has not yet adopted a good method for the determination of inorganic and organic nitrogen in fertilizers.

At the Detroit meeting of the Fertilizer Division of the American Chemical Society the following resolution was passed:

¹ *This Journal*, 1928, 11: 126.

Resolved, that the Secretary of the Fertilizer Division call the attention of the General Referee on Fertilizers of the A. O. A. C. to the official methods for determination of mineral and organic nitrogen in fertilizers, and request that these methods be further studied in order to differentiate more closely between the organic and mineral forms of nitrogen in mixed fertilizers.

The Associate Referee on Nitrogen has been studying this subject for two years and has made the recommendations previously mentioned.

The Associate Referee on Potash reports on a method for chlorine in fertilizer. His results justify the recommendation that this method be adopted as tentative. This action is especially desirable because there is no official method for this determination.

The Associate Referee on Nitrogen Activity Methods in Fertilizers made a thorough study of the alkaline permanganate method and now makes recommendations for more clearly defining the conditions for securing concordant results.

The attention of the association is called to the meeting of fertilizer manufacturers, fertilizer control officials, agronomists, and editors of farm papers, held at Louisville, Kentucky, Sept. 29-30, 1927.

The two following resolutions are of especial interest to this association:

I. The conference having agreed, in the interests of national uniformity, that the order of stating fertilizer analyses should be phosphoric acid, nitrogen and potash; that nitrogen should be stated as nitrogen and not as ammonia; and that the guarantees of phosphoric acid, nitrogen and potash should be stated only in whole numbers with no fractions:

Be it resolved that every effort be made by all the agencies concerned to put these recommendations into effect.

V. By reason of the confusion that exists in the public mind concerning the nature of the effect of the fertilizer product resulting from the action of sulfuric acid on phosphate rock because of the term "acid" that usually is placed before "phosphate" in naming this product:

Be it resolved that the matter of renaming the product be referred to the Committee on Definitions of Terms and Interpretation of Results on Fertilizers of the Association of Official Agricultural Chemists with request for action after consultation with affected interests.

The term "superphosphate" was recommended in place of acid phosphate.

REPORT ON GRAVIMETRIC DETERMINATION OF PHOSPHORIC ACID.

By WILLIAM H. ROSS (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*.

The work of the past year on the gravimetric determination of phosphoric acid was a continuation of that described in three previous reports¹ to this association. This work was undertaken in response to

¹ *This Journal*, 1925, 8: 407; 1926, 9: 182; 1927, 10: 190.

a commonly expressed view that the accuracy of the official method varies with the condition under which precipitation is made with magnesia mixture. The first two reports showed that the reaction of the solution to which the magnesia mixture is added has a small but appreciable effect on the accuracy of the method. Thus, alkaline solutions were found to give results which were a little too low and acid solutions a little too high, while best results were obtained with neutral solutions.

Other variations in the way precipitation is made with magnesia mixture, described in Lundell and Hoffman's¹ single and double precipitation methods and in Jörgensen's² method as now used in Germany, were considered sufficiently important to receive further study. Accordingly the collaborative study on the relative accuracy of these different procedures as compared with the official method was continued.

Three standard samples were selected for this year's work. Sample No. 1 was pure monopotassium phosphate. This was prepared by treating pure potassium carbonate with an equivalent amount of a highly purified sample of crystallized phosphoric acid, concentrating to crystallize out monopotassium phosphate, recrystallizing three times, and drying at 110°C. The crystals were finally ground to 100 mesh in an agate mortar.

Sample No. 2 was prepared by mixing two parts of Sample No. 1, dried to constant weight, with one part of dry potassium sulfate.

Sample No. 3 consisted of a mixture of the following materials, which are commonly associated with phosphoric acid in phosphate rock or phosphatic fertilizers:

	<i>parts</i>
Peat.....	75
Iron oxide.....	75
Fluorite	75
Calcium sulfate.....	150
Calcium carbonate	150
Kaolinite.....	225

An attempt was made to prepare Sample No. 3 as free from phosphorus as possible, but no natural materials were found that were entirely phosphorus free. A careful analysis of the sample showed a phosphoric acid (P_2O_5) content of 0.05 per cent. All the materials in the sample were ground to 180 mesh before mixing.

The directions sent to the collaborators called for the analysis of (1) Sample No. 1, (2) Sample No. 2, and (3) Sample No. 1 with an equal weight of Sample No. 3 added, by the following methods: official, Lundell and Hoffman's single and double precipitation and Jörgensen's.

¹ *This Journal*, 1924, 8: 184.

² *Analyst*, 1926, 51: 61.

DIRECTIONS FOR COLLABORATIVE WORK.

A. Determine total phosphoric acid in Sample No. 1 and Sample No. 2 by the following procedures:

(1) Dry sample at 105°C. for 1 hour. Dissolve 2 grams of the sample in water, add 5 cc. of concentrated nitric acid, and make up to the mark in a graduated flask. Transfer an aliquot of the solution into a 250 cc. beaker, add 15 grams of ammonium nitrate, and proceed as described in the official gravimetric method for the determination of total phosphoric acid until the molybdate precipitate is washed with ammonium nitrate solution. Then dissolve the precipitate on the filter with dilute ammonium hydroxide [100 cc. ammonium hydroxide (sp. gr. 0.90) per liter of water] and wash with hot water into a beaker to a bulk of not more than 100 cc. Carefully neutralize the ammoniacal solution with hydrochloric acid, using litmus paper, or brom thymol blue, as indicator; cool, and from a buret add drop by drop with stirring, 15 cc. of the official alkaline magnesia mixture for each decigram of phosphoric acid (P_2O_5) present. After 15 minutes add 12 cc. of strong ammonia. Let stand for at least 4 hours, filter through paper, and wash with dilute ammonia (100 cc. of strong ammonium hydroxide per liter) until the filter and precipitate are free from chlorides. Transfer filter and precipitate to a platinum or porcelain crucible, dry without charring, burn below redness, and ignite, preferably in an electric furnace, to constant weight at approximately 1000°C. Cool in a desiccator and weigh as magnesium pyrophosphate ($Mg_2P_2O_7$) (Official method).

(2) Prepare acid magnesia mixture as follows: Dissolve 50 grams of magnesium chloride and 100 grams of ammonium chloride in 500 cc. of water. Add ammonium hydroxide in slight excess, let stand overnight, and filter if a precipitate appears. Make barely acid with hydrochloric acid and dilute to 1000 cc.

Prepare solution of sample and proceed as in (1) through the point where the solution of the molybdate precipitate is carefully neutralized. Add 1 cc., or its equivalent, of hydrochloric acid (sp. gr. 1.19) and 20 cc. of acid magnesia mixture per decigram of phosphoric acid (P_2O_5) present. Add ammonium hydroxide (about 1 + 3) dropwise and with continuous stirring until the solution is ammoniacal and most of the phosphoric acid has been precipitated. Finally add 15 cc. more of ammonium hydroxide at one time, let stand 4 hours or overnight at room temperature, and complete the determination as in (1) (Lundell and Hoffman's modified routine method).

(3) Prepare solution of sample and proceed as in (2) through the point where the magnesium ammonium phosphate precipitate is allowed to stand 4 hours, or preferably overnight, filtered, and washed. Dissolve the precipitate on the filter in 25 cc. of dilute hydrochloric acid (1 + 1), receiving the solution in the same beaker in which the precipitation was made. Wash the filter with dilute hydrochloric acid (1 + 20) and dilute the solution to 100 cc. Add 2-3 cc. of acid magnesia mixture and then ammonium hydroxide slowly until a crystalline precipitate appears and finally an excess of 3-5 per cent by volume. Allow to stand 4-6 hours, or preferably overnight, filter on paper, wash, ignite, and weigh as in (1) (Lundell and Hoffman's umpire or double precipitation method).

(4) Prepare neutral magnesia mixture as follows: Dissolve 50 grams of magnesium chloride and 150 grams of ammonium chloride in water up to 1000 cc.

Prepare solution of sample and proceed as in (1) through the point where the molybdate precipitate is dissolved in dilute ammonia and washed with hot water into a beaker. Cover with a watch glass, bring the alkaline solution thus obtained just to the boiling point and, without cooling, add slowly from a buret drop by drop with constant stirring 20 cc. of neutral magnesia mixture per decigram of phosphoric acid (P_2O_5) present. Allow to stand without any extra addition of ammonia for at least 4 hours, filter, and complete the determination as in (1) (Jørgensen's method).

B. Determine total phosphoric acid in Sample No. 1 with an equal weight of Sample No. 3 added by the following procedures:

(1) Weigh 2 grams of Sample No. 1 into a 250 cc. beaker, add 2 grams of Sample No. 3, and digest in 30 cc. of hydrochloric acid (sp. gr. 1.19) and 10 cc. of nitric acid (sp. gr. 1.42). Cool the solution and make up to the mark in a graduated flask. Mix and pour on a dry filter or allow to settle until clear. Transfer two aliquots of the solution into 250 cc. beakers, add strong ammonia in slight excess, and barely dissolve the precipitate formed with a few drops of strong nitric acid, stirring vigorously. Add about 15 grams of ammonium nitrate and precipitate with molybdate solution as described in the official method. Complete the analysis of one aliquot as described in A (1) (official method), and the second as in A (4) (Jørgensen's method).

(2) Weigh 2 grams of Sample No. 1 and 2 grams of Sample No. 3 into a 250 cc. beaker, add 30 cc. of hydrochloric acid (sp. gr. 1.19) and 10 cc. of nitric acid (sp. gr. 1.42), and boil to a sirupy consistency. Dissolve the residue, which should be nearly solid after cooling, in 5 cc. of concentrated nitric acid and 50 cc. of water. Heat to boiling, cool, filter into a 250 cc. graduated flask, wash the filter thoroughly with cold water, and dilute to the mark. Transfer two aliquots of the solution into 250 cc. beakers, add 15 grams of ammonium nitrate, and precipitate with molybdate solution. Complete the analysis of one aliquot as described in A (2), and the second as in A (3) (Lundell and Hoffman's single and double precipitation methods).

NOTE: The presence of unburnt carbon in the ignited residue is claimed to be a source of error in the analysis of phosphates. It is possible to obtain a residue that will burn snow white throughout but under certain conditions a dark-colored residue that retains unburnt carbon after prolonged ignition may be obtained. Careful note should therefore be made if any of the procedures give a residue that fails to burn white throughout the whole mass.

ANALYSIS OF STANDARD PHOSPHATE SAMPLES.

The results reported by the collaborators are given in Table 1.

DISCUSSION.

(1) An examination of Table 1 shows that Lundell and Hoffman's single precipitation method gives results that are a little too high, but that good results are obtained with the other three methods. The mean values reported by the collaborators are essentially the same for both the official and Jørgensen's methods and show only a slight difference in favor of Lundell and Hoffman's double precipitation method. This method, however, is longer than the official method, and the difference in accuracy would seem to be too small to justify its adoption in place of the latter for routine analysis.

(2) Lundell and Hoffman's methods direct that the solution of the sample be evaporated to dryness or to a sirupy consistency to eliminate soluble silica, but this step is omitted in the official method and in Jørgensen's method. Breckenridge¹ suggested that the official method should also provide for the elimination of silica, when present, as failure to do this gives results that are a little too high. This observation is

¹ *This Journal*, 1926, 9: 186.

TABLE 1.
Results of analysis of standard phosphate samples.
(Expressed in percentage.)

COLLABORATOR	SAMPLE NO. 1.—52.18 % P_2O_5 PRESENT				SAMPLE NO. 2.—34.79 % P_2O_5 PRESENT				SAMPLE NO. 1 WITH SAMPLE NO. 3 ADDED.—52.23 % P_2O_5 PRESENT			
	Official method	Lundell and Hoff- mann's single precipi- tation method	Lundell and Hoff- mann's double precipi- tation method	Jürgen- sen's separation method	Official method	Lundell and Hoff- mann's single precipi- tation method	Lundell and Hoff- mann's double precipi- tation method	Jürgen- sen's separation method	Official method	Lundell and Hoff- mann's single precipi- tation method	Lundell and Hoff- mann's double precipi- tation method	Jürgen- sen's separation method
C. Ahrens, Dr. Gilbert's öffentl. Chem. Laboratorium, Germany	52.64	52.28	52.18	35.01	34.79	34.98	52.66	52.26	52.31
A. H. Allen, Virginia-Carolina Chemical Corporation	52.02	51.94	51.96	51.90	35.03	35.00	35.06	34.94	51.94	51.76	51.73	51.96
H. R. Allen, Kentucky Agricultural Experiment Station	52.18	52.33	52.15	52.16	34.67	34.83	34.65	34.65	52.27	52.40	52.18	52.26
J. F. Bradley, Pennsylvania Dept. of Agriculture	52.54	52.62	52.50	52.48	34.58	34.67	34.60	34.69	52.65	52.80	52.59	52.78
J. E. Breckenridge, American Agricultural Chemical Co.	52.32	53.34	52.24	52.40	35.08	35.30	35.14	52.64
E. W. Cowan, University of Missouri	52.79	52.31	52.18	52.88	35.46	35.02	34.89	35.21	52.82	52.06	51.02	53.12
J. H. Elder, Department of Agriculture, Virginia	52.40	53.00	52.00	52.09	34.75	35.26	34.59	35.24	52.75	53.00	52.57	52.52
F. O. Lundstrom, Bureau of Chemistry and Soils	52.01	52.62	52.49	52.59	34.63	35.06	35.01	35.29	52.41	51.57	51.52	52.57
A. Retter, Schilmann & Bene, Germany	52.32	52.47	52.17	52.20	35.03	35.15	34.92	34.94	52.48	52.60	52.35	52.35
D. S. Reynolds, Bureau of Chemistry and Soils	51.99	52.26	51.91	52.02	34.78	34.94	34.62	34.70	52.22	52.42	51.70	52.27
P. McG. Shuey, Savannah, Georgia	52.39	52.80	52.20	52.32	35.10	35.08	34.88	35.05	52.40	52.95	52.15	52.35
Mean	52.29	52.57	52.19	52.29	34.91	35.03	34.80	34.98	52.45	52.42	52.01	52.45
Mean of results within 0.5% of theoretical	52.24	52.40	52.19	52.23	34.85	35.03	34.80	34.98	52.37	52.32	52.26	52.32

supported by the results given in Table 1, which show that the mean value given by the official method in the analysis of Sample No. 1 is a little too high when Sample No. 3 is added.

(3) A review of the literature indicates that much of the controversy over methods of phosphoric acid analysis has been due to the use of standards of variable composition or of materials that have been standardized by chemical analysis alone. The work of the past year shows that monopotassium phosphate, or a mixture of this salt with potassium sulfate, makes an excellent standard for phosphate analysis. It crystallizes readily and is now prepared in a high state of purity for use as a buffer in hydrogen-ion work. It has no water of crystallization and can be readily dried to constant weight at 110°C. without danger of decomposition. It is also one of the least hygroscopic of soluble salts.

(4) The magnesia mixture used in the official method is alkaline while that used in Jörgensen's method and in Lundell and Hoffman's methods is either neutral or slightly acid. The use of alkaline magnesia mixture has the disadvantage that it readily becomes contaminated through its action on the glass container and so is likely to prove a source of error in phosphate analysis. This condition may be avoided by preparing a supply of neutral magnesia mixture and then adding ammonia only to that portion of the mixture required for immediate use.

(5) The report presented last year showed that errors of analysis may also arise from the presence of unburnt carbon in the ignited residues. It was found that failure to obtain white residues may be due to the manner of igniting the precipitates rather than to any preceding step in the determination and that determinations in which the residues do not burn white are unreliable. Precipitates that have been properly prepared readily burn to snow whiteness throughout when ignited in an electric muffle furnace at 1000°C.

Recent investigations by Jacob and Reynolds¹ also show that the loss of phosphorus from an intimate mixture of carbon and magnesium pyrophosphate is insignificant below a temperature of 1000°C., but that the loss may be considerable above this temperature. This emphasizes the importance of first burning at a low temperature to destroy most of the carbon and then completing the ignition at a higher temperature.

ACKNOWLEDGMENT.

The associate referee wishes to express his appreciation to the collaborators who have cooperated so faithfully in the work from year to year and particularly to P. McG. Shuey for helpful suggestions and for securing the cooperation of the German laboratories in this year's collaborative work.

¹ *This Journal*, 1928, 11: 128.

RECOMMENDATIONS¹.

It is recommended—

(1) That the present phase of the collaborative study of the gravimetric determination of phosphoric acid be discontinued.

(2) That the words *nearly neutralize with strong hydrochloric acid* in the gravimetric determination of phosphoric acid (7)² be changed to read *neutralize with strong hydrochloric acid, using litmus paper or brom thymol blue as indicator* (first action).

(3) That the words *burn first at a low heat and then ignite intensely until white or grayish white* in the gravimetric determination of phosphoric acid (7)² be changed to read *burn first at a low heat and ignite to constant weight, preferably in an electric furnace, at approximately 1000°C.* (first action).

(4) That the words *dilute to 1 liter* in the second of the alternative methods given for the preparation of magnesia mixture [5 (c) (2)]³ be changed to read *proceed as in (1)* (final action).

(5) That a third alternative method for the preparation of magnesia mixture [5 (c)] be worded to read as follows:

(3) Dissolve 55 grams of crystallized magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in water, add 140 grams of ammonium chloride, and dilute to 870 cc. Add strong ammonium hydroxide to each required portion of the solution just before using at the rate of 15 cc. per 100 cc. of solution (first action).

REPORT ON NITROGEN.

By A. L. PRINCE⁴ (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*.

The problem of finding a suitable method for the accurate determination of nitrate nitrogen in mixed fertilizers containing cyanamide and urea was further considered by the associate referee during the present year. Last year's work on the Jones method⁵ yielded some promising results, but before this study was completed it was thought advisable to investigate a number of other methods that appeared to have possibilities.

The Busch nitron method⁶ was tried out on some freshly prepared samples. The principle of this method consists in precipitating the nitrate as nitron nitrate from a water extract by means of the reagent "nitron acetate". The precipitate forms quantitatively and may be

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 68.

² *Methods of Analysis*, A. O. A. C., 1925, 3.

³ *Ibid.*, 2.

⁴ Presented by R. N. Brackett.

⁵ *Ind. Eng. Chem.*, 1927, 19: 269.

⁶ Treadwell and Hall. *Analytical Chemistry*, Vol. 2, 1924, p. 391; *Ber.*, 1905, 38: 861; *Z. angew. chem.*, 1905, 13: 494.

washed, dried, and weighed. The method was found to be accurate and comparatively simple even in the presence of compounds like cyanamide and urea. However, the high cost of the reagent "nitron" eliminated the method as one that could be used in routine or control work. It could be used to advantage, however, in checking the analyst's work occasionally or on special problems.

The Arnd method¹ was also brought to the attention of the associate referee. This method is quite similar in principle to the Devarda method² but, like the Devarda method, it can be used for nitrate salts alone, and not in the presence of organic materials. It consists of the reduction of the nitrate salts in a neutral aqueous solution by means of hydrogen produced with a special alloy (40 per cent magnesium and 60 per cent copper) in a magnesium chloride solution. Although the method is simple and seems to yield accurate results when used on nitrate salts alone, it is the opinion of the associate referee that it possesses no distinct advantage over the Devarda method, which has already been adopted as a tentative method.

The Busch nitron and the Arnd methods being eliminated, there remained the Jones method for the determination of nitrates in the presence of cyanamide and urea, mentioned previously. Since the results obtained last year by 20 collaborators were much closer to theoretical than those obtained by the present official method³, further work with this method was planned.

Two samples of mixed fertilizers were carefully prepared. Sample No. 1 was of the formula 5-9.38-6. The sources of nitrogen were as follows: nitrate of soda, sulfate of ammonia, tankage, and calcium cyanamide. The latter was added in a quantity equivalent to 41.3 pounds per ton. Sample No. 2 corresponded to the formula 5-9.57-6. The mixture was similar to No. 1 except that urea was used in place of calcium cyanamide. The latter was added in quantity equivalent to 17.7 pounds per ton. In both samples, the theoretical quantity of nitrogen as nitrate was 1.65 per cent, and of nitrogen as ammonia, 1.24 per cent. It will be noted that these samples contained very small quantities of cyanamide and urea. Since last year's samples contained excessive quantities of these substances, it was important to learn whether or not similar differences in analyses by the two methods occurred when small quantities of cyanamide and urea were present. The samples were sent out to fifteen chemists. Thirteen reports were received.

¹ *Z. angew. chem.*, Aufsatzteil 1, 1917, 30: 169.

² *Methods of Analysis*, A. O. A. C., 1925, 12

³ *Ibid.* 11

INSTRUCTIONS TO COLLABORATORS.

AMMONIACAL NITROGEN.

Magnesium Oxide Method—Official.

The directions for this method are the same as those given in 33, p. 11, *Methods of Analysis*, A. O. A. C., 1925, with one exception: Use a sample of 1 gram instead of 0.7–3.5 grams.

NITRIC AND AMMONIACAL NITROGEN.

Reduced Iron Method—Official.

Same as 34, p. 11, *Methods of Analysis*, A. O. A. C., 1925, with two exceptions: Use a sample of 1 gram instead of 0.7 or 1 gram and add 10 cc. of dilute sulfuric acid instead of 20 cc.¹

JONES METHOD FOR DETERMINING NITRATE NITROGEN IN FERTILIZERS
CONTAINING CYANAMIDE, UREA, ETC.

This method has been published².

NOTES.

- (1) To prevent caking, add water to the flasks at the completion of the process, before cooling and washing.
- (2) The addition of perforated glass beads insures gentle boiling, free from bumping.
- (3) From 50 to 60 minutes is usually required for the distillation of A and B.
- (4) During distillation the flasks should rest on asbestos-coated wire gauze.

EXPERIMENTS.

Series I. Run each sample for ammoniacal nitrogen in triplicate by the magnesium oxide method (official) as described.

Series II. Run each sample for nitric nitrogen in triplicate by the reduced iron method (official) as described. Subtract the ammoniacal nitrogen to obtain the nitric nitrogen.

Series III. Run each sample for ammoniacal nitrogen in triplicate by the Jones method as described.

Series IV. Run each sample for nitric nitrogen by the Jones method as described. Each determination is made in two parts (A and B). Run in duplicate or in triplicate if time permits.

The collaborators were the following:

1. L. S. Walker, Massachusetts Agricultural Experiment Station, Amherst, Mass.
2. L. A. Salinger, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Savannah, Ga.
- 3 and 4. W. D. Richardson, Swift and Co., Chicago, Ill.
5. M. P. Etheredge, Mississippi State Chemical Laboratory, A. & M. College, Miss.
6. T. A. Balthis, Division of Chemistry, Department of Agriculture, Richmond, Va.
7. L. C. Norem, Armour and Co., Chicago, Ill.
8. W. E. Thrun, Agricultural Experiment Station, East Lansing, Mich.
9. A. O. Olson, Dairy and Food Department, St. Paul, Minn.
10. L. J. Jenkins, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.
11. J. E. Breckenridge, American Agricultural Chemical Co., Carteret, N. J.
12. G. F. Anderson, Agricultural Experiment Station, Burlington, Vt.
13. A. L. Prince.

¹ NOTE: There is an error in the last edition (1925) of *Methods of Analysis*: "20 cc. of dilute sulfuric acid (1 + 1)", should read "10 cc. of dilute sulfuric acid (1 + 1)".

² *This Journal*, 1927, 10: 198; 1928, 11: 32.

DISCUSSION.

A summary of the results showing the averages obtained by both methods by each collaborator is given in the accompanying table. Columns 1 and 2 record the results of the ammonia nitrogen on both samples by the official and Jones methods. The data indicate that the two methods give approximately the same results, the general averages on Sample No. 1 being the same, 1.25 per cent. The same was true of Sample No. 2, the general average being 1.23 per cent by both methods. These results were anticipated since this section of the Jones method (determination of ammonia nitrogen) was only a slight modification of the official method. However, the data are important, because if the Jones method were used for the nitrate determination, it would be advantageous to use the complete Jones method and thereby save time. It is apparent from these collaborative results that this could be done.

Under column 3 are recorded the results of the nitrate nitrogen by the official method, and under column 4 by the Jones method. With Sample No. 1, which contains calcium cyanamide, it is quite obvious that the results by the official method are distinctly higher than those obtained by the Jones method. Furthermore, the results by the Jones method come nearer to the theoretical value in most cases. On Sample No. 1, the general average by the official method is 1.72, or 0.07 per cent above the theoretical value, and by the Jones method 1.62, or only 0.03 per cent below the theoretical value.

The same tendency holds true on Sample No. 2, which contained urea, although the differences between the results by the two methods are not so marked. Apparently the urea is not so easily broken down in the analytical process as cyanamide. In last year's samples¹, where excessive quantities of cyanamide and urea were present, the variations between the two methods were proportionately greater. Therefore, it is important to note that the same condition exists when smaller quantities of these substances are present in the mixed fertilizer.

As pointed out in last year's work, the reason for the high results when using the official method is believed to be due to the action of the hydrogen evolved from the iron and sulfuric acid on the organic materials. In the Jones method the action of the hydrogen on these materials is offset by the use of a concentrated soda solution. In part B of the analysis, the sulfuric acid and concentrated soda balance the effect of the iron, sulfuric acid, and soda treatment of part A on the organic materials. In this way, part B of the Jones method serves the purpose of obtaining a proper correction blank.

In the associate referee's report for 1925², it was recommended that the zinc-iron method be placed under the heading, "Nitrogen in Nitrate

¹ *This Journal*, 1927, 10: 196.

² *Ibid.*, 1926, 9: 191.

Salts", as it is unsuitable for mixed fertilizers. However, in view of the fact that many chemists find this method confusing and undesirable, and also because there are several other official methods that meet the same needs, it seems advisable at this time to discard the zinc-iron method. The Devarda method and the reduced iron method fully meet all the requirements for which the zinc-iron method was originally intended.

It is also desirable that the reduced iron method be marked "applicable only in the absence of cyanamide and urea"; otherwise, it would appear that the reduced iron method could be used for nitrates under any conditions.

Averages of collaborative results.

(Expressed as percentage of nitrogen.)

COLLABORATORS	AMMONIA NITROGEN THEORETICAL VALUE, 1.24 %		NITRATE NITROGEN THEORETICAL VALUE, 1.65 %	
	Official Method	Jones Method	Official Method	Jones Method
SAMPLE No. 1.				
1	1.30	1.27	1.65	1.68
2	1.18	1.16	1.80	1.62
3	1.22	1.26	1.72	1.61
4	1.28	1.33	1.58	1.59
5	1.28	1.30	1.74	1.65
6	1.25	1.36	1.66	1.61
7	1.14	1.14	1.77	1.59
8	1.29	1.26	1.66	1.65
9	1.35	1.33	1.78	1.43*
10	1.24	1.18	1.72	1.54
11	1.20	1.15	1.68	1.68
12	1.32	1.26	1.67	1.54
13	1.26	1.22	1.81	1.64
Average	1.25	1.25	1.72	1.62
SAMPLE No. 2.				
1	1.29	1.29	1.62	1.60
2	1.08	1.06	1.83	1.66
3	1.20	1.19	1.69	1.56
4	1.18	1.21	1.67	1.66
5	1.27	1.30	1.64	1.65
6	1.30	1.35	1.57	1.63
7	1.19	1.22	1.71	1.66
8	1.22	1.31	1.65	1.68
9	1.35	1.28	1.56	1.60
10	1.28	1.25	1.68	1.60
11	1.32	1.15	1.56	1.65
12	1.19	1.24	1.71	1.59
13	1.18	1.12	1.72	1.59
Average	1.23	1.23	1.66	1.63

* Omitted from average.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the Jones method for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide or urea be adopted as a tentative method.
- (2) That the zinc-iron method be discarded.
- (3) That the reduced iron method be marked "applicable only in the absence of cyanamide and urea".

REPORT ON NITROGEN ACTIVITY METHODS IN FERTILIZERS.

DETERMINATION OF ACTIVE WATER-INSOLUBLE NITROGEN BY THE ALKALINE PERMANGANATE METHOD².

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.),
Associate Referee.

The formal study of nitrogen activity methods, discontinued by this organization in 1916, was resumed largely because of dissatisfaction with the lack of uniformity in results reported by different analysts using the alkaline permanganate method³.

Experience has shown that an analyst accustomed to this method can obtain concordant results and that this is also true in regard to several workers in the same laboratory who use the same apparatus and follow the same procedure, but that difficulty arises when results obtained in different laboratories are compared. The obvious conclusion is that the method, as now written, allows sufficient variation at important points to cause a significant discrepancy in the data obtained, and that the correction lies in a more exact wording of the directions. The work here reported, therefore, deals entirely with this method and with but one aspect of the problem, that of securing such uniformity in the procedure that more concordant results will be obtained.

The work has been further restricted to consideration of this method as applied to mixed fertilizers, since such appears to be its chief use at present. Collaborative work was not attempted as the first need seemed to be a study of the different points in preparation for such work in the future.

MATERIALS.

As time was not available for an exhaustive study of all types of mixed fertilizers, it seemed advantageous to use an approximate average of all the types. Therefore, each sample was prepared by compositing portions

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 68.

² Contribution No. 360 of the Rhode Island Agricultural Experiment Station.

³ *Methods of Analysis*, A. O. A. C., 1925, 12.

of six samples of complete mixed fertilizers selected at random from those that had been drawn during the previous year from the stocks of commercial dealers in Rhode Island. These samples were then passed through a 1 mm. sieve and thoroughly mixed. Differences due to the various treatments of these average samples should hold for all fertilizers, but they would probably be of less magnitude than those found for materials of greater individual variation.

PRELIMINARY WORK.

Determination of water-insoluble nitrogen.

It was observed in a paper by Magruder¹ that like results for water-insoluble nitrogen are not always obtained in collaborative work. The cause of such discrepancy did not, however, appear from an investigation of the more obvious points of deviation from the directions. As shown in Table 1, the results for water-insoluble nitrogen were in satisfactory agreement despite the following variations in technic: Wash water at 20°C. versus 25°C. and acid added to dry versus wet residue in Kjeldahl flask. Nor was this consistency disturbed by the use of ten different brands of filter paper varying in texture, size, and time required for passage of 250 cc. of water through the sample (Table 2). High results were shown, however, from such an inexcusable departure as failure to complete the washing by not using the whole 250 cc. of water designated. The determination of the blank due to nitrogen in the filter paper proved important because one brand consistently showed a significant quantity when several sheets from the package were examined. This blank should be determined only after washing from the paper any soluble nitrogen that may be due to ammonia or nitric acid absorbed from the air.

Preparation of a washed residue containing 50 mg. of water-insoluble nitrogen.

The directions provide for weighing a sample calculated to contain 50 mg. of water-insoluble nitrogen on a 11 cm. filter paper and washing with 250 cc. of water at room temperature. This procedure was compared with one previously advocated by Jones² in which it was specified that amounts of more than 4 grams be weighed into a small beaker and washed a few times by decantation before being transferred to the filter for completion of the washing. Nitrogen was determined in the residues. Results obtained by the second method are not significantly lower than those obtained by the first procedure (Table 1). The second procedure is advocated, however, because it is considered more appropriate for the washing of a large quantity of material with a relatively small quantity of water.

¹ *This Journal*, 1922, 5: 455.

² *J. Ind. Eng. Chem.*, 1912, 4: 438.

The residues were dried on the filters at a temperature below 80°C. and transferred to the 600 cc. Kjeldahl flask by the use of a spatula and a small, stiff brush, care being taken to remove as few paper fibers as possible. Nitrogen was determined in the material transferred, also the nitrogen that remained on and in the paper. The greatest deviation from the desired 50 mg. of nitrogen was a shortage of 2.1 mg. For insoluble nitrogen having an activity of 75 per cent, this would result in an error of 4.2 per cent. For extreme accuracy the quantity of nitrogen actually transferred to the Kjeldahl flask should be determined by washing duplicate portions of sample, transferring them to flasks as usual, and determining total nitrogen in one and active nitrogen in the other. This wise provision has appeared in certain previous outlines of the method¹, but it does not seem advisable to transfer the filter with the residue for the total nitrogen determination as there advocated, since the nitrogen left in the paper and in the unremoved residue was found to be well above 1 mg. (Table 1). Such nitrogen would not be included in the actual determination of active nitrogen.

DIGESTION AND DISTILLATION.

Detailed discussion of the factors involved in digestion and distillation has been left for a later section of this report. It was found that approximately 60 per cent of the active nitrogen was distilled in the first 15 cc., 20 per cent rather equally distributed over the succeeding 60 cc. and 20 per cent in the final 20 cc. Five per cent of the nitrogen distilled was recovered in the last 5 cc. of distillate. It is apparent that the required volume of 95 cc. of distillate must be measured accurately and that the distillation must be stopped promptly at this point.

The quantity of ammonia left in the condensers and recovered by subsequent washing of the tubes averaged the equivalent of 0.4 per cent of active nitrogen in seventeen trials. The method is purely definitive and does not allow the inclusion of the nitrogen that remains in the condenser tubes. This ammonia was disregarded, but it was removed after completion of the distillation by allowing 100 cc. of water to be drawn through each tube by the suction of the cooling flask. No blank was discovered in several distillations of the reagents alone.

The preliminary work did not indicate significant differences from variations in time and temperature of digestion or from the use or omission of 0.2 gram of paraffin. There was, however, a decided effect due to rate and time of distillation. In fifty carefully controlled distillations an average of 18 cc. of distillate was collected in the first 15-minute period, 24 cc. in the second, 27 cc. in the third, and 26 cc. in the last. This is approximately a uniform rate of distillation for the 60-minute period adopted.

¹ *J. Ind. Eng. Chem.*, 1912, 4: 438.

DEMONSTRATION OF THE RELATIVE EFFECTS OF VARIOUS DEVIATIONS FROM AN ADOPTED PROCEDURE FOR DIGESTION AND DISTILLATION.

As originally planned, a modification of the official method was written in accordance with the findings reported above, and the results obtained by this method were used as a basis to demonstrate the relative magnitudes of errors caused by certain deviations from the directions.

The method adopted was as follows:

PROCEDURE.

Place a quantity of material equivalent to 50 mg. of water-insoluble nitrogen on a smooth-surface filter paper and wash with water at room temperature until the filtrate measures 250 cc. If more than 4 grams is required, weigh into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed. Dry the residue at a temperature not exceeding 80°C. and transfer from the filter to a 500-600 cc. Kjeldahl distillation flask, removing adhering particles by rubbing lightly with a stiff brush. Avoid the inclusion of filter-paper fibers. Add 20 cc. of water, 5 cc. of small perforated glass beads, and 100 cc. of alkaline permanganate solution. Attach to an upright condenser so arranged as to conduct the distillate through a glass tube to a point below the surface of an accurately measured quantity of standard acid contained in a 100 cc. graduated cylinder. Digest for 30 minutes in a bath maintained at a temperature of 110°C., rotating the flask gently at intervals to prevent material from adhering to the flask above the surface of the liquid. Remove the bath and place the flask in an asbestos ring having a circular opening 2.5 inches in diameter in the center. Raise the temperature and distil uniformly at such a rate that approximately 24 cc. passes over in each 15 minute period, keeping the material in contact with the liquid by rotating the flask frequently. As the distillate collects in the receiving cylinder, lower the cylinder or support it at an angle and draw it away from the condenser, keeping the delivery tube barely below the surface of the distillate. The volume of distillate may be determined by comparison with a similar cylinder placed at the same angle, filled with water to the level of the liquid in the first and returned to an upright position. Collect 95 cc. of distillate in 60 minutes, plus or minus 5 minutes. Transfer the contents of the cylinder to an Erlenmeyer flask and titrate with standard alkali, using cochineal indicator. The nitrogen thus obtained is the active water-insoluble nitrogen.

To eliminate any lack of uniformity in the quantities of water-insoluble nitrogen placed in the flasks for the various treatments, the water-insoluble nitrogen was washed from a number of portions of the sample in accordance with the usual procedure, and the dried residues from each sample were combined, passed through a 1 mm. sieve, and thoroughly mixed. The weight of the combined sample divided by the number of residues included gave the quantity of material to be weighed into the flasks to furnish 50 mg. of water-insoluble nitrogen.

The various modifications attempted and the results obtained are reported in Table 3.

DISCUSSION OF RESULTS.

From the data in Table 3 it may be seen that duplicate determinations, conducted on different days, may be expected to agree within 1 per cent, or the equivalent of a 2 per cent error on material showing an activity of 50 per cent.

The use of a piece of paraffin the size of a pea and weighing 0.2 gram did not affect the results materially, although Samples 1 and 5 show minus errors of less than 4 per cent. The same two samples were similarly affected by the use of a small drop, 0.02 gram, of mineral lubricating oil. These low results may possibly be ascribed to the reduction of a small quantity of permanganate by the paraffin or oil, as discussed by Moore and White¹. Either reagent is of decided value in preventing frothing and makes possible a uniform rate of distillation. This advantage is considered sufficient to offset the small losses of nitrogen activity noted. Preference is given to the lubricating oil because it neither collects on the walls of the condenser nor clouds the filtrate as does paraffin.

Extension of the digestion period to 1 hour, as allowed in the official method, did not significantly affect the results, but a reduction of the temperature from 110°C. to 100°C. gave slightly lower activities. A 30-minute digestion period should be sufficient for mixed fertilizers, especially if lubricating oil is used to eliminate frothing.

Consistent differences were noted from variations in both the time and rate of distillation. Lengthening the period to 90 minutes increased the average nitrogen activity of the six samples by 5 per cent, while shortening the period 45 minutes decreased it by an equal amount. Either of these modifications is allowable under the official directions, although Jones, the author of the method, has commented as follows: "One hour is usually sufficient for the distillation of 95 cc., and it is recommended that as nearly as possible 90 minutes be taken for digestion and distillation". It is an important factor in obtaining uniform results.

It was also found that results could be varied at will by changes in the rate of distillation at different periods within the stipulated 60 minutes. Thus, rapid distillation at the start, followed by slow distillation until completion of the 95 cc. required at the end of 1 hour, gave results that averaged 6 per cent below those found for the reverse of this process. This difference is illustrated in Fig. 1. The discrepancy is probably due to a longer exposure of the protein material to a greater concentration of alkali and to a higher degree of heat in the first instance. Both these factors would increase the rate of protein hydrolysis and aid in a greater production of ammonia. Both the rate and time of distillation should be carefully controlled, and this is easily possible when some reagent, as oil or paraffin, is used to prevent frothing.

In all the work reported here, the escape of any undissolved ammonia fumes from the condenser was prevented by conducting the distillate below the surface of the liquid in the receiving cylinder. Omission of this precaution, that is allowing the distillate to drop from a delivery

¹ *This Journal*, 1926, 10: 202.

² *J. Ind. Eng. Chem.*, 1912, 4: 438.

tube extending barely 1 inch within the mouth of the cylinder, resulted in an average loss for six distillations of 11.7 per cent of the ammonia. Some adequate method of trapping undissolved fumes should be employed.

EFFECT OF THE CONCENTRATION OF POTASSIUM PERMANGANATE IN THE ALKALINE PERMANGANATE SOLUTION ON THE ACTIVITY OF THE WATER-INSOLUBLE NITROGEN.

Alkaline solutions of potassium permanganate are subject to decomposition on standing, as is evidenced by the appearance of a green color, probably due to the formation of a manganate. After standing for 60 days, such a solution produced less active nitrogen under the conditions of the method than did a fresher solution (Table 3).

To test this point further, each of three samples was treated with four different solutions of alkaline permanganate differing in age and in method of preparation. All solutions were prepared by stirring 25 grams of C. P. potassium permanganate in hot water and 150 grams of C. P. sodium hydroxide in cold water, combining the two solutions when cold, and diluting to 1000 cc. Solution I was used when more than 60 days old, solution II when 10 days old, and solution III and IV when but 2 days old. Solution III was made from crystals of potassium permanganate that passed a 1 mm. sieve, while those used for solution IV were too large to pass through a 2 mm. sieve. The oxidizing power of each solution was determined by titration with oxalic acid in the presence of an excess of sulfuric acid. The oxalic acid had been compared with especially purified sodium oxalate.

The results obtained are reported in Table 4. A correlation may be observed between the oxidizing power of the solution and the quantities of active nitrogen found. The potassium-permanganate equivalent of the solutions was surprisingly low, but whether this condition was due to actual decomposition of the permanganate, failure to completely dissolve the reagent, or to the method used in its estimation is not clear. The finer salt did not prove superior to the coarser. A fifth solution, made by dissolving with greater care a double quantity of potassium permanganate, showed 50.08 grams of the reagent before dilution with a sufficient quantity of double-strength sodium hydroxide (300 grams per liter) to reduce the concentration by one-half. The potassium-permanganate equivalent of the diluted solution after 4 days was found to be but 24.10 grams per liter as compared with the 25.04 grams that was expected.

Time was not available for a more thorough investigation of the alkaline permanganate solution, but it seems essential to make certain of the complete solution of the potassium permanganate by the use of heat. The titration and adjustment of the permanganate solution previous to the

addition of the solution of sodium hydroxide is strongly recommended. Solutions showing evidence of decomposition should be discarded.

RECOMMENDATIONS¹.

(1) The following changes in official methods, *Methods of Analysis*, A. O. A. C., 1925, are recommended:

Page 12, 38, line 3: Insert "water-insoluble" before the word "nitrogen". The passage will then read, "making a correction for the water-insoluble nitrogen of the filter, if necessary".

Page 12, 40: Change to read, "Dissolve 25 grams of potassium permanganate in hot water and, separately, 150 grams of sodium hydroxide in cold water; combine the solutions when cold and dilute to 1 liter. Discard permanganate solutions that have become green in color".

Page 13, 41 (a): After line 3, add "When it is found necessary to use 4 or more grams of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed".

Page 13, 42, line 3: After the sentence, "transfer from the filter to a 500-600 cc. Kjeldahl distillation flask", add the phrase, "loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers".

Page 13, 42, line 4: In place of "a piece of paraffin the size of a pea", substitute the following: "a drop of lubricating oil of medium viscosity". Line 5: Change the sentence, "Connect with, etc.", to read as follows: "Connect with an upright condenser to the lower end of which has been attached a 100 cc. graduated cylinder containing standard acid, and so arranged as to receive the distillate below the surface of the acid or otherwise so trapped as to prevent loss of ammonia fumes". Line 7: Omit the words "at least" and change to read, "barely below distillation point". Line 9: Change the sentence, "Distil until 95 cc. of distillate is obtained", to read, "Distil 95 cc. in 60 minutes (plus or minus 5 minutes), controlling the distillation so that approximately 24 cc. of distillate is obtained in each 15 minute period. Conduct the first part of the distillation over a bare flame but use wire gauze 10 minutes before completion to avoid breaking the flask". Also before the direction to "Titrate with standard alkali" add the direction, "transfer the distillate to a flask or to a beaker". After the last line add, "If the active water-insoluble nitrogen is found to be less than 55 per cent of the total water-insoluble nitrogen present, it is recommended that a second portion of the sample be prepared as directed under 41 (a). Dry the residue below 80°C., transfer from the filter to a Kjeldahl flask as directed above, and determine nitrogen as directed under 19 or 22. Recalculate the percentage of active water-insoluble nitrogen on the basis of the quantity of water-insoluble nitrogen thus found".²

(2) It is also recommended that the study of the nitrogen activity methods be continued with a view to discovering:

- a. The effect of different degrees of fineness in material ground to pass a 1 mm. sieve.
- b. The appropriateness of stricter standards for the alkaline permanganate solution used.
- c. The application of the method to mixtures containing uric acid.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 69.

² Par. 42, amended according to these recommendations, has been published. See *This Journal*, 1928, 11: 34.

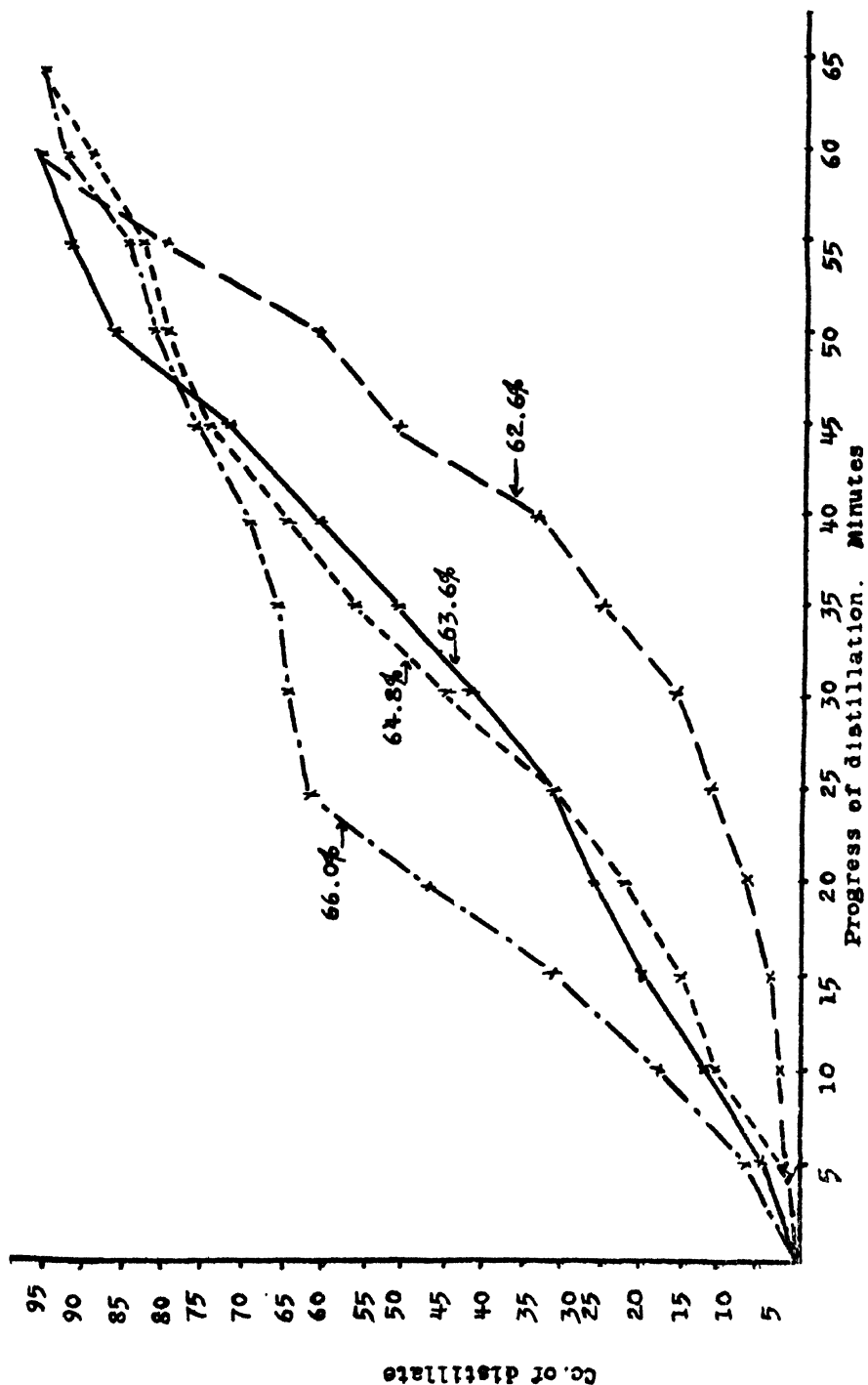


FIG. 1. EFFECT OF RATE OF DISTILLATION ON THE PERCENTAGE OF ACTIVE WATER-INSOLUBLE NITROGEN FOUND IN MIXED FERTILIZERS.

TABLE 1.

Determination of the water-insoluble nitrogen of mixed fertilizers, and the probability of placing 50 mg. of such nitrogen in a distillation flask by the official method.

Sample No.....	WATER-INSOLUBLE NITROGEN					
	1	2	3	4	5	6
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Extracted with water at 20°C.....	0.64	0.36	0.87	0.62	0.68	1.43
Extracted with water at 25°C.....	0.63	0.43	0.87	0.63	0.65	1.46
Residue digested without previous drying	0.63	0.40	0.87	0.63	0.60	1.47
Average.....	0.63	0.40	0.87	0.63	0.64	1.45
Extraction with less than 250 cc.....		0.49*		0.61†		1.54‡
Nitrogen in residue after extracting with 250 cc. of water the amount of sample calculated to contain 50 mg. of water-insoluble nitrogen.						
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Extracted on filter.....	51.5	49.2	50.0	50.7	51.4	53.2
Nitrogen in residue and filter.						
Washed by decantation, then on filter...	51.1	48.3	50.7	50.1	50.9	52.7
Nitrogen in residue and filter.						
Washed as above, brushed into flask. . . .	51.2	47.9	48.8	49.4	50.0	50.1
Nitrogen in residue without filter.						
Nitrogen not removed from filter in above transfer.....	1.6	1.5	1.5	1.3	1.5	1.4

* 230 cc. † 240 cc. ‡ 215 cc.

TABLE 2.

Effect of different types of filter paper on the determination of the water-insoluble nitrogen of mixed fertilizers.

TYPE OF PAPER	WATER-INSOLUBLE NITROGEN*	TIME OF WASHING†	NITROGEN BLANK FROM PAPER‡
	<i>per cent</i>	<i>minutes</i>	<i>per cent</i>
E. & A. 11 cm. qualitative.....	1.46	50	0.06
B. & A. 11 cm. qualitative.....	1.43	35	0.02
C. S. & S. 11 cm. quantitative.....	1.47	25	0.01
Whatman No. 2, 11 cm. qualitative.....	1.44	70	0.01
Whatman No. 50, 11 cm. hardened.....	1.44	130	0.03
C. S. & S. No. 575, 11 cm. hardened.....	1.41	50	0.02
C. S. & S. No. 2, 12.5 cm. qualitative.....	1.43	70	0.01
E. & A. 12.5 cm. qualitative.....	1.43	25	0.01
C. S. & S. 9 cm. qualitative.....	1.46	55	0.00
C. S. & S. Blue band 9 cm. quantitative.....	1.47	130

* After subtracting blank due to filter paper.

† Required for passage of 250 cc. of water through sample and paper.

‡ Includes only water-insoluble nitrogen in the filter paper.

TABLE 3.

Effect of variations in the technic of digestion and distillation.

Sample No.....	Average	ACTIVITY OF WATER-INSOLUBLE NITROGEN					
		1	2	3	4	5	6
Official method, modified*.....		62.2	58.8	65.0	59.8	71.6	65.2
		63.0	59.8	66.2	60.8	71.4	66.4
RELATIVE QUANTITIES OF ACTIVE NITROGEN OBTAINED, BASED ON AVERAGE OF ABOVE RESULTS AS 100							
0.2 gram paraffin added†.....	99.1	96.4	99.7	100.3	101.9	96.9	99.4
One drop of lubricating oil, medium viscosity.	98.1	94.5	100.3	99.1	99.8	96.6	98.1
Digested 60 minutes†.....	98.4	98.8	96.7	101.0	97.8	101.2	98.4
Digested at 100°C.†.....	97.0	96.2	99.4	95.9	96.9	96.4
Distilled in 90 minutes†.....	105.2	103.4	107.6	104.4	106.4	102.3	107.2
Distilled in 45 minutes†.....	94.6	92.6	95.5	91.3	96.0	96.9	95.3
Distilled slowly for 30 minutes, then rapidly to completion. Total time, 60 minutes.†	96.9	97.5	100.4	96.7	98.1	96.9	91.9
Distilled rapidly for 30 minutes, then slowly to completion. Total time, 60 minutes.†	102.8	100.3	103.5	103.5	105.0	101.9	102.8
Ammonia fumes in distillate not trapped†. Water passing through the condensers at 12°C.	88.3	84.5	93.1	82.0	94.3	83.7	92.2
Alkaline permanganate solution 60 days old. Green in color.†	96.0	90.9	96.2	96.2	99.8	98.1	95.0

* Digest with perforated glass beads for 30 minutes at 110°C. Distil 95 cc. in 60 ± 5 minutes so that approximately 24 cc. is obtained in each 15 minute period. Distillate conducted under the surface of standard acid in a 100 cc. graduated cylinder.

† Allowed by the official method.

TABLE 4.

Effect of variations in the oxidizing power of alkaline permanganate solutions.

Alkaline permanganate solution.....	1*	2†	3‡	4§
Oxidizing power¶ of the solution expressed in terms of potassium permanganate in acid solution (<i>grams per liter</i>).....	21.5	22.3	22.5	22.5
ACTIVITY OF WATER-INSOLUBLE NITROGEN				
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Sample A.....	55.9	58.7	60.8	58.4
Sample B.....	59.6	62.2	62.9	63.0
Sample C.....	66.0	67.2	69.1	68.9
Average.....	60.5	62.7	64.2	63.4

* More than 2 months old, green in color.

† 10 days old.

‡ 2 days old. Made from potassium permanganate that passed a 1 mm. sieve.

§ 2 days old. Made from potassium permanganate that failed to pass a 2 mm. sieve.

¶ Determined by titration with oxalic acid in the presence of an excess of sulfuric acid. The oxalic acid was equivalent to especially purified sodium oxalate.

NOTE.—In preparing each solution, the potassium permanganate had been dissolved in hot water.

REPORT ON POTASH.

By A. P. KERR (Agricultural Experiment Station, Baton Rouge, La.),
Associate Referee.

The method of determining potash in commercial fertilizer by eliminating phosphorus from the solution prior to evaporation requires further study; therefore, the finished report is not ready at this time.

DETERMINATION OF CHLORINE IN COMMERCIAL FERTILIZERS.

Two methods for the determination of chlorine were offered for study. In one the sample was made alkaline by adding calcium carbonate in order to drive off any ammonia present before washing on the filter. In the other the sample was washed on the filter with distilled water and titrated direct. The direct titration method seems to be more practical, and therefore the results by this method only are reported.

The samples were composed of the following fertilizer materials:

Sample No. 1—Acid phosphate, cyanamide, cottonseed meal, potassium chloride.

Sample No. 2—Acid phosphate, sodium nitrate cyanamide, potassium chloride.

Sample No. 3—Acid phosphate, sodium nitrate, ammonium sulfate, potassium chloride.

Sample No. 4—Acid phosphate, ammonium sulfate, cyanamide, potassium chloride.

The following method was used:

Results of analysis obtained by using direct titration method.

COLLABORATORS	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3	SAMPLE NO. 4
	3.14 %	3.18 %	1.78 %	1.63 %
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
W. F. Hand A. & M. College Miss.	3.37	3.19	1.64	1.72
R. Page Hudson Department of Agriculture Richmond, Va.	3.21	3.18	1.49	1.64
W. Catesby Jones Department of Agriculture Richmond, Va.	3.28	3.01	1.50	1.57
L. D. Haigh University of Missouri Columbia, Mo.	3.35	3.19	1.43	1.74
E. S. Asbury A. & M. College Texas	3.36	3.27	1.69	1.70
J. W. Sample Department of Agriculture Nashville, Tenn.	3.40	3.19	1.56	1.70
J. L. Farr Agricultural Experiment Station Baton Rouge, La.	3.20	3.29	1.80	1.72

REAGENTS.

(a) *Standard silver nitrate solution*.—Dissolve about 5 grams of pure recrystallized silver nitrate in distilled water and make up to 1000 cc. Standardize against pure, dry sodium chloride. 1 cc. = 0.001 gram of chloride.

(b) *Potassium chromate solution*.—Dissolve 5 grams of potassium chromate in 100 cc. of distilled water.

PROCEDURE.

Weigh 2.5 grams, place on a 11 cm. filter paper, and wash into a 250 cc. flask with successive portions of boiling water. Cool, make to mark, and pipet 50 cc. into a 150 cc. beaker. Add 1 cc. of potassium chromate and titrate with standard silver nitrate solution until a permanent red color of silver chromate is formed.

RECOMMENDATION.

It is recommended that this method be further studied, and that other materials than those found in the mixtures given this year be used¹.

No report on plants was given owing to the resignation of the referee. The following paper was substituted.

DETERMINATION OF IRON AND ALUMINUM IN THE PRESENCE OF CALCIUM, MAGNESIUM, AND PHOSPHORIC ACID.

By A. J. PATTEN and O. B. WINTER² (Michigan Experiment Station,
E. Lansing, Mich.).

In 1923 Patten³ reported a method for the separation of iron and aluminum from calcium in the presence of phosphoric acid by precipitating the iron and aluminum as phosphates while keeping the solution within a definite range of hydrogen-ion concentration. This method is useful to the plant chemist because it eliminates the process of removing the phosphoric acid from the solution before making the iron and aluminum determinations, and therefore the necessity of handling a large volume of solution.

Although this same procedure was used by Fiske⁴ and by Stadie and Ross⁵ for the removal of phosphates from solutions, and the method as a whole has been used extensively, the following questions have been raised regarding its accuracy:

1. What effect has the reaction of the solution on the precipitation of the salts in question?

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 71.

² Published with the permission of the Director of the Experiment Station as Journal Article No. 43 from the Chemical Laboratory.

³ *This Journal*, 1923, 6: 418.

⁴ *J. Biol. Chem.*, 1922, 51: 55.

⁵ *Ibid.*, 1925, 65: 735.

2. How effective is the buffer which is used to control this reaction?
3. Are the ferric and aluminum phosphates completely precipitated?
4. What is the effect of washing the iron and aluminum precipitates with hot 5 per cent ammonium nitrate solution?

It is the purpose of this paper to give some of the data obtained in this laboratory which bear upon these questions. The technic followed in an attempt to extend the method to make possible microchemical separations and determinations of iron and aluminum will also be discussed.

The method now in use in this laboratory differs from the original Patten method in a few minor details. It is as follows:

Have the iron in the ferric state and remove the silica. If the solution does not already contain an excess of phosphate, add to an aliquot containing approximately 40 mg. of ferric and aluminum phosphates 0.5 gram of diammonium hydrogen phosphate, stir until dissolved, and make up to 50 cc. with distilled water. Add a few drops of thymol blue and then add ammonium hydroxide until the solution just turns yellow. Run in 0.5 cc. of concentrated hydrochloric acid, follow with 25 cc. of 25 per cent ammonium acetate, and stir. Let stand at room temperature until the precipitate settles (approximately 1 hour). Filter and wash ten times with hot 5 per cent ammonium nitrate solution. Ignite and weigh as ferric and aluminum phosphates.

1. EFFECT OF THE REACTION OF THE SOLUTION ON THE PRECIPITATION OF THE SALTS.

No question having arisen in regard to the precipitation of the ferric and aluminum phosphates under the conditions specified, there remain to be considered the hydrogen-ion concentration at which calcium phosphate precipitates and the efficiency of the buffer in holding the reaction of the solution below this concentration.

As shown by electrometric curves in Patten's original article, the calcium began to precipitate at a pH of about 6.5. To check this result the following two experiments were performed:

1. Five solutions containing iron, aluminum, and calcium were adjusted to reactions of pH 4.3, 4.7, 5.4, 6.0, and 6.7. The iron and aluminum were precipitated, filtered, and washed by the method described previously in this paper, and the precipitates were redissolved and tested for calcium by the McCrudden method¹. The results showed calcium present only in the precipitate from the solution of pH 6.7, whereas the iron and aluminum precipitated in all the solutions.

2. Synthetic solutions containing iron, aluminum, and calcium were prepared, and the iron, aluminum, and calcium determinations were made as stated in the preceding paragraph. The results of these analyses are given in Table 1.

The results in Table 1 indicate that no appreciable amount of calcium is carried down with the iron and aluminum.

¹ *J. Biol. Chem.*, 1909, 7: 83.

TABLE 1.
Results of determinations of iron, aluminum, and calcium.

SOLUTION	FePO ₄ -AlPO ₄ THEORETICAL gram	Ca THEORETICAL gram	FePO ₄ -AlPO ₄ FOUND gram	Ca FOUND gram
1	0.0939	0.0075	0.0932 0.0936	0.0077 0.0081
2	0.0469	0.0151	0.0454 0.0472	0.0077 0.0150 0.0153 0.0151

2. EFFICIENCY OF THE BUFFER.

Theoretically, the statement that the addition of ammonium acetate may raise the pH of the solution above the point at which calcium phosphate will be precipitated is not true if practically pure ammonium acetate is used, and the chemically pure grades found on the market appear to be perfectly safe in this respect. To test this point, solutions were prepared from three different brands, and each was found to have a pH of approximately 5.4. This value, then, represents the upper limit to which the ammonium acetate solution can raise the reaction of any solution to which it may be added. This is a safe point. Furthermore, several solutions from which the iron and aluminum had been precipitated according to the same method were tested and were invariably found to have reactions between pH 5.1 and 5.3.

The effectiveness of the buffer is further shown by the following additional experiment:

Fifty cubic centimeters of distilled water was acidified with a small quantity of dilute hydrochloric acid. The solution was neutralized to thymol blue (acid range) with dilute ammonium hydroxide, and 25 cc. of 25 per cent ammonium acetate was added. To this solution concentrated hydrochloric acid was added in varying quantities, the resulting pH values being as follows:

HCl cc.	pH
0	5.4
0.5	5.2
1	5.1

These data show that ammonium acetate is a good buffer for that part of the pH range in which ferric and aluminum phosphates are precipitated (5.0-5.4) and that the reaction of the solution in every case will undoubtedly fall within the range in which the iron and aluminum phosphates may be separated from the calcium phosphate. Therefore, the ammonium acetate solution is a suitable buffer for holding the solutions within this required range, even if some concentrated hydrochloric acid is added after neutralizing the solution to thymol blue.

3. COMPLETENESS OF THE PRECIPITATION OF FERRIC AND ALUMINUM PHOSPHATES.

To show the completeness of the precipitation of the phosphates, iron

and aluminum determinations were made on several synthetic ferric and aluminum chloride solutions, both separately and combined, by the same method and also by precipitating them as the hydroxide and burning to the oxide. In Table 2 the results of these analyses will be seen. Column (a) gives the theoretical amount of the element present, column (b) the amount determined as the oxide¹ and calculated to the element, and column (c) the amount determined as the phosphate and calculated to the element.

TABLE 2.

Results showing completeness of precipitation of phosphates.

SOLUTION		(a)	(b)	(c)
		gram	gram	gram
Iron.....	1	0.0052	0.0053 0.0054 0.0056	0.0054
	2		0.0264 0.0262	0.0268 0.0264
	3		0.0272 0.0267	0.0275 0.0278
Aluminum.....	1	0.0161	0.0162 0.0160	0.0161 0.0159
	2	0.0141	0.0141 0.0143	0.0143 0.0144
	3	0.0225	0.0228 0.0225 0.0222	0.0226 0.0222 0.0221
Iron and aluminum	1	0.0361		0.0374
	2	0.0248		0.0245
	3	0.0480		0.0478

From the data given in Table 2 it will be seen that the iron runs slightly higher when determined as the phosphate than it does when determined as the oxide. When determined by the two methods, the aluminum runs practically the same. These results, and also qualitative tests on the filtrates from these determinations, indicate that the precipitation is complete in both cases.

4. EFFECT OF WASHING THE IRON AND ALUMINUM PRECIPITATES WITH HOT 5 PER CENT AMMONIUM NITRATE SOLUTION.

It has been found necessary in the method described to wash the ferric and aluminum phosphate precipitates about ten times with *hot* 5 per cent ammonium nitrate solution. In carrying out this procedure the wash solution should be nearly boiling, and the precipitate should be thoroughly loosened from the filter and broken up as completely as possible each time with a fine spray from a wash bottle. In order to ascertain whether

¹ Olsen. Quantitative Chemical Analysis, 4th ed., p. 181.

this excessive washing with the hot solution rendered soluble or hydrolyzed the phosphates, iron and aluminum were precipitated as phosphates from several synthetic solutions. These precipitates were then washed as directed above, burned, weighed as the phosphates, and calculated to the elements. The results are recorded in Table 3.

TABLE 3.
Results showing solubility of precipitates.

	SOLUTIONS	WASHINGS	THEORY GRAM	FOUND GRAM
Iron	1	10	0.0265	0.0267
				0.0269
		20		0.0263
				0.0259
		30		0.0257
				0.0255
Aluminum	1	10	0.0161	0.0164
				0.0158
		20		0.0155
				0.0152
				0.0151
		30		0.0152
				0.0153
				0.0156
	2	10	0.0142	0.0143
				0.0144
		25		0.0139
				0.0140
	3	10	0.0147	0.0143
				0.0143
				0.0143
				0.0141
		30		0.0141
				0.0142

It appears from the results given in Table 3 that both iron and aluminum phosphates are slightly soluble in hot 5 per cent ammonium nitrate solution. These data, together with those in Table 2, which show that when the iron is determined as the phosphate it has a tendency to run slightly higher than the theoretical amount present, make it appear that the loss due to solubility when the precipitates are washed ten times, as above stated, is compensated by another error that causes the iron to run high.

In analyzing plants for iron and aluminum in this laboratory, this method has been found more satisfactory than any other that has been tried. The data presented in the tables are typical of the results that have been obtained, and it is believed that they justify the use of the method. However, one disadvantage is the large quantity of material that must be used for making the determinations and even then the amounts present at the final weighings are too small for the best results. In view of this fact an attempt was made to apply a microchemical method for precipitating the iron and aluminum as phosphates and for the

separation and determination of the iron and aluminum. The technic of this method is similar to that used by Mull, Morrison, and Myers¹ for determining aluminum in blood and is carried out as follows:

Oxidize the iron to the ferric state, remove the silica, and acidify the solution until it contains an excess of at least 1 cc. of 6 *N* hydrochloric acid. Measure an aliquot of the solution containing about 1 mg. of ferric and aluminum phosphate into a centrifuge tube having a capacity of about 25 cc. and with a mark at 20 cc. Add water to make a volume of about 10 cc. Then add about 0.1 gram of ammonium hydrogen phosphate (unless there is a sufficient amount of phosphate present to precipitate all the iron and aluminum) and shake until dissolved. Add a drop of thymol blue and then ammonium hydroxide until the solution just turns yellow. Add 1 cc. of saturated ammonium acetate solution and let stand at room temperature until the precipitate partially settles (approximately 30 minutes). Centrifuge, decant the supernatant liquid, wash once with 3 cc. of 5 per cent ammonium nitrate solution, and again centrifuge and decant. Now dissolve the precipitate by adding 1 cc. of 6 *N* hydrochloric acid and dilute to about 10 cc. with water. Add 5 cc. of 6 *N* potassium hydroxide and 2 cc. of acetic acid (1 + 2), heat in a steam bath for 20 minutes, cool, and centrifuge. (The precipitate contains the iron, and the supernatant liquid, the aluminum.) Decant the supernatant liquid, wash with a few cc. of water, and again centrifuge and decant.

After separating the iron from the aluminum, determine the iron colorimetrically as follows: Dissolve the precipitate obtained as directed in the preceding paragraph in 2 cc. of 6 *N* hydrochloric acid and add water to bring to the 20 cc. mark. Place an aliquot in a 50 cc. graduated flask, add water to make about 35 cc. and sufficient hydrochloric acid to give 2 cc. of 6 *N* acid in the sample, cool to about 15°C., add 5 cc. of 15 per cent ammonium sulfocyanate, fill to the mark, shake, and compare with a standard.

The investigation for the determination of aluminum is being continued. It is based on the colorimetric method described by Mull, Morrison, and Myers, to which reference has been made. The ammonium salt of aurin tricarboxylic acid (aluminon) is used as the dye for developing the color.

Several solutions containing iron and aluminum were treated according to this microchemical method, and the supernatant liquids were tested for these elements. After the iron and aluminum separations were made, several iron precipitates were dissolved and tested for aluminum, and the solutions containing the aluminum were tested for iron. The tests for iron were made with ammonium sulfocyanate; those for aluminum were made with the spectograph by O. S. Rask of the School of Hygiene of Johns Hopkins University, to whom the thanks of the writers are due. In each case only slight traces of aluminum were found. Similar results were obtained in the examination of the ammonium nitrate washings.

This colorimetric method for the determination of iron has been in use for a long time, and as applied to this work it gives satisfactory results. Nearly all the determinations fall within a range of 4 per cent error.

¹ Paper read by title before the Iowa Branch of the Society of Experimental Biology and Medicine, Feb. 2, 1927.

The colorimetric method for the determination of aluminum, however, is not entirely satisfactory. It has been developed so that many of the results fall within a range of 5 per cent error, which is the degree of accuracy desired. Allowance must be made for the fact that nearly all the reagents used in these methods contain a small quantity of both iron and aluminum.

SUMMARY.

The data given confirm the conclusions drawn in the earlier paper on this same subject and show that—

1. Ferric and aluminum phosphates are precipitated from solutions below pH 5.3, while calcium is not precipitated until a pH of about 6.5 is reached.

2. Ammonium acetate is a suitable buffer for keeping the reaction of the solution within the range necessary for separating ferric and aluminum phosphates from calcium phosphate.

3. Ferric iron and aluminum may be completely precipitated as phosphates.

4. When iron is determined as the phosphate, the results run slightly high. The increase, however, is practically within experimental error.

5. Ferric and aluminum phosphates are slightly soluble in hot 5 per cent ammonium nitrate solution. When washed ten times, however, the loss is practically compensated by the slight increase in the weight of the iron phosphate.

6. A microchemical method is discussed for precipitating and separating iron and aluminum as phosphates and for determining the iron and the aluminum colorimetrically.

REPORT ON PREPARATION OF PLANT MATERIAL FOR ANALYSIS.

By H. R. KRAYBILL (Agricultural Experiment Station, Purdue, Ind.),
Associate Referee.

In *Methods of Analysis* the paragraph dealing with the preparation of sample of any plant material for analysis mentions only the method of air drying. In many of the studies with plants this method is not satisfactory, especially where the various forms of carbohydrates and nitrogenous compounds are determined. Considerable work has been done by various investigators in making a study of the reliability of such methods as the preservation in alcohol, the freezing of the material, and the drying of the material at different temperatures. The associate referee has compiled this information and feels that more work is needed before any recommendation except of general character can be made.

RECOMMENDATION¹.

It is recommended that study on the preparation of plant material for analysis be continued.

No report on the less common metals in plants was given by the associate referee.

REPORT ON TOTAL CHLORINE IN PLANTS.

By DORIS H. TILDEN (Food, Drug and Insecticide Administration, San Francisco, Calif.), *Associate Referee*.

With the growing importance of the determination of the inorganic elements of plants, the need for an accurate method for determining chlorine in plant and food products has been felt.

The Carius method² is probably the best for determining chlorine in organic material, but it is obviously too cumbersome and slow for plant or food analysts. Therefore methods involving incineration have been generally adopted. These, however, are not uniform and are often unsatisfactory. Browne³ states that a considerable loss of this element (chlorine) occurs even under the most careful methods of incineration. If the quantities of chlorine are comparatively small, as in most plant products, the ease with which it volatilizes, especially in the presence of sulfur and phosphorus, according to Filippo and Adriani⁴, and even in the presence of carbon alone, as reported by Drost⁵, causes the errors in determining this element after direct ashing to be excessive. When Browne and Gamble⁶ incinerated various chlorides with sugar, they found a loss of 100 per cent with magnesium chloride, 94 per cent with calcium chloride, 37 per cent with potassium chloride, and 34 per cent in the case of sodium chloride. Drost⁵ reported a loss of 22-33 per cent of chlorine on heating a mixture of sodium and potassium chloride with pure lactose, and Dill⁷ found that a large and variable loss occurs in the sodium chloride content of clams when they are ashed without preliminary addition of a binding agent.

EXPERIMENTAL WORK.

To test the previous statements, experiments were made to determine the loss of chlorine that might occur when sodium chloride is ashed with

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 71.

² *Ann.*, 1865, 136: 129.

³ *This Journal*, 1926, 9: 19.

⁴ *Z. Untersuch. Lebensm.* 1926, 51: 374.

⁵ *Ibid.*, 375.

⁶ *Facts About Sugar*, 1923, 17: 552.

⁷ *This Journal*, 1925, 8: 447.

pure sucrose under varying conditions. The results obtained have been summarized in Table 1.

TABLE 1.

Losses of chlorine occurring when sodium chloride is ashed with sucrose.

SUGAR ADDED	CHLORINE PRESENT	CHLORINE RECOVERED (GRAVIMETRIC METHOD)	LOSS
<i>grams</i>	<i>mg.</i>	<i>mg.</i>	<i>per cent</i>
10	60.65	45.91	24.3
10	30.32	8.95	70.48
10	15.16	0.69	95.4
10	15.16	0.00	100.0
5	60.65	48.19	20.5
5	45.45	37.11	18.4
5	30.32	23.72	21.77
5	15.16	14.52	4.22
2.5	60.65	49.30	18.7
2.0	15.16	10.27	32.2

It is quite apparent that the relative quantities of carbon and chlorine materially affect the percentage loss of chlorine from salt. When the quantities of the element present are small, large quantities of carbon will cause a heavy loss of chlorine. In one case 10 grams of sucrose at muffle temperature caused the complete volatilization of 15 mg. of chlorine. Temperature probably also affects the extent of the interaction of sodium chloride and carbon. That there is such an interaction was shown by the alkaline character of the residue in all cases. The associate referee had no opportunity to determine whether carbonate formation was equivalent to loss of chlorine, but she corroborates the statements of the authorities quoted and is convinced that a method for chlorine determination involving direct ashing of the material is too unreliable and unaccurate for an A. O. A. C. method.

Filippo and Adriani, Drost, and Browne and Gamble recommend chlorine methods that do not involve incineration. Such procedures, however, do not take into consideration the effect of colloidal or pectinous substances possibly present, which may cause the silver chloride to precipitate in a colloidal form and so prevent quantitative handling. The associate referee found that the addition of a quantity of pectin, approximately equal to the amount present in fruits, to a dilute nitric acid solution of sodium chloride, equivalent to the average quantity of chlorine in fruits, caused, on the addition of silver nitrate, the formation of a colloidal silver chloride that did not settle completely after standing 48 hours. It was in such a state of subdivision that it could not be retained on a Gooch prepared for the regular silver chloride precipitate.

There is the further possibility that plants may have organic or combined chlorine, which would be lost by a direct method of determination. The silver chloride may also occlude or adsorb organic or coloring matter.

Colloids and adsorbed organic matter, therefore, may cause errors of opposite sign in direct gravimetric determinations, while coloring matter will usually prevent the use of volumetric methods. In Table 2 are given data obtained by the associate referee on the chlorine content of assorted foods to show the general unsatisfactory character of the methods involving ashing without fixatives and direct precipitation.

TABLE 2.

Comparison of methods of preparing foods for gravimetric chlorine determinations.

PRODUCT	DIRECT ASHING	ASHED IN PRESENCE OF 500 MG. OF Na_2CO_3	DIRECT DETERMINATION IN SAMPLE SOLUTION ACIDIFIED WITH HNO_3
<i>Chlorine, mg. per 100 grams</i>			
H_2O extract of raspberry	8.09	11.38	9.08
H_2O extract of raspberry	3.96	9.90	4.28
H_2O extract of raspberry		17.64	11.70
H_2O extract of orange pulp		16.15	5.86
H_2O extract of figs.	13.93	19.97	16.65
H_2O extract of pomegranate		50.3	Went through Gooch
Pomegranate juice*	30.83	52.46	Went through Gooch
Pineapple juice*	57.6	63.4	56.56
H_2O extract of quince	11.8	14.3	
H_2O extract of strawberry†	9.66	31.1	Went through Gooch
H_2O extract of tomato pulp†	25.7	22.89	27.96
H_2O extract of tomato pulp†	29.45	28.45	26.72
H_2O extract of tomato puree†	47.5		41.82
H_2O extract paste stock† (tomato)	46.1		34.57
H_2O extract of ripe tomato†	26.70	24.87	26.06
<i>Chlorine, percentage of sample</i>			
Prepared mustard	0.917	0.93	Went through Gooch
Cheese	0.33	1.13	
Oysters	1.73	3.39	
H_2O extract of tomato catsup	2.02	2.04	1.89
H_2O extract of tomato catsup	2.08		1.17
H_2O extract of tomato paste	0.56		0.49

* Milligrams of chlorine per 100 cc.

† Determinations made by J. C. Palmer, Seattle Station.

Of the various foods examined, tomato products only seem to show no loss of chlorine owing to direct ashing, but the reason has not been satisfactorily explained. With one exception the results by the direct method have also been uniformly lower than those obtained by ashing in the presence of sodium carbonate. The gains due to adsorbed coloring matter (in some cases the silver chloride was much discolored) did not counterbalance the losses due to colloids and possibly to organic chlorine. In many cases the colloidal nature of the silver chloride was so pronounced that no chlorine determination, however inaccurate, was possible. It appears, therefore, that in many, if not in all cases, the food analyst must resort to methods involving ashing in the presence of fixatives.

OFFICIAL GRAVIMETRIC METHOD.

At the outset of the work, the official gravimetric method for the determination of chlorine was used. It involves precipitating the chlorine from a boiling solution with silver nitrate and continuing the boiling till the precipitate settles. Inconsistencies, however, soon became apparent for it was difficult to recover, consecutively, theoretical quantities of chlorine from known solutions. In many cases the weight of the silver chloride was entirely too high, and its duplicate was too low. The conditions under which precipitation occurred, the solubility of silver chloride, and the possibilities of occlusion or adsorption were suggested explanations of these difficulties. Therefore a search of the literature was made for details of methods that would produce correct and consistent results.

As reported by Seidell¹ the solubility of silver chloride per liter of water increases from 1.5 mg. at 20°C. to 22 mg. at 100°C., and it is also dependent upon the degree of dispersion of the precipitate. A flocculent is more soluble than a granular precipitate, according to Drucker², owing, perhaps, to the difference in size of the grains formed. The more compact, less soluble precipitate obtained either by heating or by long standing in contact with the mother liquor, loses to a great extent the possibility of being thoroughly washed. This may be explained by the ease with which silver nitrate is occluded. Richards and Wells³ note that Stas found that in working with solutions of silver nitrate weaker than 0.1 *N* occlusion was so slight as to be negligible. They conclude that continued shaking of the cold silver chloride-silver nitrate solution would *diminish* the solubility of silver chloride as well as *disengage* the occluded salt. They worked usually at a temperature of 20°C., filtered the silver chloride as soon as possible after the supernatant liquor became clear, and washed the precipitate by decantation with three portions of 0.2 liter each of dilute silver nitrate solution, then with cold water. The same procedure was followed by Richards and Willard⁴ in their revision of the atomic weight of silver, except that a cold nitric acid wash and ice-cold water were used.

Besides the gravimetric method for determining chlorides, the official methods recognize Volhard's volumetric thiocyanate method. Although more rapid than a gravimetric determination it has been considered inaccurate for small quantities of chlorine. The Gay-Lussac method⁵ for silver may be applied to the determination of chlorine, and the possibility of combining the Gay-Lussac volumetric method with the gravimetric method to obtain a double check is also strongly recommended.

¹ Van Nostrand's Chemical Annual, 4th issue, 1918, p. 223.

² Z. Chem. Ind. Kolloide, 1909, 4: 216.

³ Carnegie Institution of Washington, Pub. No. 28, 1905.

⁴ Ibid., 125, 1910.

⁵ Scott. Standard Methods of Chemical Analysis, 4th ed., vol. 2, p. 457.

COMPARISON OF METHODS.

In order to compare these three methods as to accuracy, speed, and ease of manipulation, a series of experiments was performed on a standard solution of sodium chloride prepared from chemically pure salt recrystallized and dried. A standard silver nitrate solution was made by dissolving an accurately weighed quantity of pure-proof silver (better than 999 fine) in a small quantity of nitric acid, boiling the solution to remove oxides of nitrogen, and making up to volume with distilled water. An approximately 0.05 *N* solution was prepared in this manner and used throughout all subsequent experiments. In the Volhard method a 0.05 *N* thiocyanate solution was used for the back titration. In anticipation of the use of a fixative for chlorine in subsequent ashing experiments, 500 mg. of chemically pure sodium carbonate was uniformly added to the sodium chloride solution and also sufficient nitric acid to make the solution decidedly, but not strongly, acid.

The Gay-Lussac and gravimetric determinations were made on the same aliquot. To the solution diluted to about 200 cc. and contained in a glass-stoppered Erlenmeyer flask, the major part of the 0.05 *N* silver nitrate reagent required was added quickly from a buret, the flask was stoppered and wrapped in a black cloth, and the solution was agitated by shaking. The precipitate gathered quickly into more or less granular masses and settled rapidly. The last portions of the precipitant were added in very small quantities, the amount of precipitate formed at each addition being a guide to the quantity to be added next time. The flask was placed on a piece of black paper during the addition of silver nitrate, in order to observe the precipitate more easily. After each addition of precipitant, the flask was covered, agitated, and set aside in a dark place. When the last two drops of silver nitrate failed to produce a precipitate, the previous reading of the buret was recorded, and the chlorine was calculated. To the solution in the Erlenmeyer flask was then added 1 cc. excess silver nitrate reagent. After the precipitate had settled completely, the solution was decanted through a weighed Gooch, and the precipitate was washed by decantation with cold water and finally transferred to the crucible as rapidly and with as little exposure to light as possible. The total volume of washings and mother liquor usually amounted to from 750 cc. to 1 liter. The silver chloride was then dried at 140°–150°C., weighed, and calculated to chlorine.

For the Volhard method the aliquot was placed in a glass-stoppered volumetric flask, the size varying with the quantity of chlorine expected. The standard silver nitrate solution was added in excess, and the flask was briskly shaken until the precipitate settled clear. The mixture was made up to volume with distilled water and filtered on a dry filter paper. An aliquot equivalent to half the initial volume was titrated with 0.05 *N* thiocyanate solution, ferric alum being used as indicator. This depar-

ture from the regulation Volhard method was thought justified owing to the difficulty in washing the coagulated silver chloride clean and to the very small volume occupied by the precipitate. The results obtained with these three methods are given in Table 3.

TABLE 3.

Comparison of the gravimetric, Gay-Lussac, and Volhard methods for the determination of known quantities of chlorine.

(500 mg. of Na_2CO_3 added in all cases.)

QUANTITY CHLORINE PRESENT	QUANTITY CHLORINE DETERMINED		
	Gay-Lussac Method	Gravimetric Method	Volhard Method
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
121.3	121.68	122.09	121.90
121.3	121.2	121.8	120.7
100.4	100.55	100.0	100.45
60.65	60.64	60.77	60.60
60.65	60.65	60.66	60.71
60.65	60.67	60.52	60.32
60.65	60.67	60.45	60.32
60.65	60.64
60.65	61.26
60.65	60.71
50.2	50.40	50.28	50.16
30.32	31.00	30.30	29.73
30.32	30.47	30.72	29.77
30.32	30.29	30.82	30.76
25.10	25.07	24.62	25.06
15.16	15.31	15.21	15.29
15.16	15.31	15.26	15.29
15.16	15.15	14.57	15.29
10.04	11.79	9.87	10.69
10.04	10.92	9.97	10.48
6.06	6.06	5.63	6.30
6.06	5.97	6.35	6.32
3.03	3.11	2.79	3.57
3.03	3.11	2.72	3.54
3.03	3.11	2.47

It will be observed that the agreement in results obtained by the three methods is remarkably close and that consistent checks with the theoretical amounts of chlorine present are obtained when very small as well as large quantities are to be determined. The three methods appear about equally accurate although they are not equally rapid. The Volhard method is by far the most rapid. It is evident also that errors due to occlusion of silver or sodium nitrates and the solubility of silver chloride have been almost entirely eliminated. The associate referee now felt justified in continuing the search for an effective fixative for chlorine during incineration.

The official methods recommend in several instances that sodium carbonate be used as a binding agent for chlorine in the ashing process. Barium hydroxide and calcium acetate have also been suggested. The

associate referee made some experiments to determine the effectiveness of these reagents, using pure sucrose, and sodium chloride as the most stable salt of chlorine and magnesium chloride as the least stable¹. The results obtained are recorded in Table 4.

TABLE 4.

Effectiveness of chlorine fixatives under varying conditions.

FIXATIVE	SUGAR PRE- SENT	CHLORINE PRESENT AS NaCl	CHLORINE RECOVERED			CHLORINE PRESENT AS MgCl ₂	CHLORINE RECOVERED	
			Gay- Lussac Method	Gravi- metric Method	Volhard Method		Gravi- metric Method	Volhard Method
	<i>grams</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Calcium acetate <i>mg.</i> 500	10	59.64			57.99	40.16	18.83	
Barium hydroxide <i>mg.</i> 500	10	60.65			39.19	56.91	48.68	
Sodium carbonate <i>mg.</i>								
100	10	60.65		54.10				
200	10	60.65		58.10				
300	10	60.65		59.12				
300	10	60.65		59.95	60.43			
400	10	60.65		60.39				
400	10	60.65		60.49	60.18			
500	10	139.16			139.00	50.2		49.18
500	10					100.4		96.23
750	10	139.16			138.60	50.2		49.35
1000	10	139.16			138.10	50.2		49.46
500	10	121.3		118.8				
500	10	121.3	118.26	117.7	120.2			
500	10	60.65		61.76				
500	10	60.65		61.20	60.80			
500	10	60.65			60.50			
500	10	30.32			30.34			
500	10	15.16			15.08			
500	10	6.06			6.16			
500	5	121.3	118.9	118.2	115.0			
500	5	60.65			60.34			
500	5	30.32	29.53	29.68	30.73			
500	5	15.16	15.17	14.92	14.87			
500	5	6.06			6.02			
500	2	121.3	120.1	119.9	112.2			
500	2	15.16			15.75			
500	2	6.06			5.95			
250	10	60.65	58.73	58.24	53.21			
250	10	30.32			28.57			
250	10	15.16			15.19			
250	5	60.65			61.14			
250	5	15.16			14.99			
250	2	60.65			61.14			
250	2	15.16			15.26			
250	1	60.65			60.51			
250	1	15.16			15.26			

¹ *Facts About Sugar*, 1923, 17: 552.

Some interesting results are recorded in Table 4. They indicate that sodium carbonate is the superior fixative and that calcium acetate and barium hydroxide when present in the same proportions as sodium carbonate fail to hold the chlorine satisfactorily. This condition might be expected in the light of the results of Browne and Gamble, which show that sodium chloride is the most stable salt of chlorine in the presence of hot carbon. The results also show that sodium carbonate in large excess is required to prevent loss by volatilization. If the loss of chlorine is represented by the reaction $2 \text{NaCl} + \text{C} + 3\text{O} = \text{Na}_2\text{CO}_3 + 2 \text{Cl}$, the law of mass action would require excess sodium carbonate to prevent the reaction from going toward the right. There also appears to be a rough relationship between the relative quantities of carbon and chlorine present, and the quantity of sodium carbonate required to fix the chlorine. For example, 250 mg. of sodium carbonate is sufficient to fix 60 mg. of chlorine in the form of sodium chloride provided the organic matter does not exceed the equivalent of 5 grams of sugar. When the sugar is increased to 10 grams, 250 mg. of the carbonate will successfully hold about 15 mg. of chlorine, but it appears insufficient to prevent some volatilization if the chlorine is increased to 30 or more milligrams. Five hundred milligrams of sodium carbonate will fix approximately 100 mg. of chlorine in the form of sodium chloride, but that quantity seems to be about the limit. Chlorine in the form of magnesium chloride is harder to fix, but 500 mg. of sodium carbonate seems to do fairly well even if the chlorine amounts to 100 mg. and the sugar to 10 grams. The chlorine content of plant products in the amounts usually taken for analysis rarely exceeds 100 mg., and the addition of 500 mg. of sodium carbonate before ashing apparently allows a remarkably efficient recovery of chlorine after incineration.

METHODS.

The working method that has proved satisfactory to the referee for determining chlorine in plant material is as follows:

PREPARATION OF SAMPLE.

To the sample, or an aliquot of a sample solution contained in a platinum dish, add 500-800 mg. of C. P. sodium carbonate in solution. Thoroughly incorporate the material with the sodium carbonate solution. evaporate to dryness, and char at a low temperature in a muffle. If necessary, add small quantities of water from time to time to expose new carbon surfaces, evaporate, and further heat in the muffle in order to get a white ash. (To avoid loss by decrepitation, it may be advisable to add a few cubic centimeters of alcohol to the dried residue and burn this off before reincineration.) Dissolve the ash carefully in approximately 50 per cent nitric acid and determine chlorine by the Gay-Lussac, gravimetric, or Volhard method.

PROCEDURE FOR GAY-LUSSAC METHOD.

Filter the nitric acid solution of the ash into a 750 cc. glass-stoppered Erlenmeyer flask, wash the filter thoroughly, and dilute if necessary to about 200 cc. with distilled

water. Add from a buret an accurately standardized silver nitrate solution of a strength not over 0.1 *N*, and preferably about 0.05 *N*. (The silver nitrate solution may be added rapidly at first, but not to excess.) Stopper the flask, cover with a black cloth, and shake the solution violently after each addition until the precipitate becomes granular and leaves a clear supernatant liquid. When the precipitate becomes light, use great care to avoid passing the end point. Near the end of the titration hold the flask over a black glazed paper to aid in the detection of the end point. When the last two drops of the silver nitrate solution fail to produce a precipitate, take the previous reading as final and calculate the chlorine directly from the value of the standard solution.

NOTE.—This method requires much time toward the end of the determination, owing to the necessity of allowing the silver chloride to settle completely after each addition of silver nitrate and also to the small quantity of precipitant that may be added each time.

PROCEDURE FOR GRAVIMETRIC METHOD.

If desired, follow a Gay-Lussac determination by a gravimetric determination. In such cases add about 1 cc. of silver nitrate in excess, shake, and filter after the solution has become clear. When a Gay-Lussac determination is not desired, proceed as for that method to the addition of dilute silver nitrate (not in excess of 0.1 *N* strength). Add the silver nitrate solution to *slight excess*, stopper the flask, cover it with a black cloth, and shake until the precipitate becomes granular and leaves an almost clear supernatant liquid. Allow to stand in the dark until clear, and then decant through a weighed Gooch prepared with a thin pad of asbestos and previously dried at 140°–150°C. Wash by decantation several times, using in all 500 or 600 cc. of *cold* distilled water. Transfer the precipitate to the crucible with cold water, dry first at 100°C. and finally at 140°–150°C., weigh, and calculate the weight of silver chloride to chlorine. (The silver chloride becomes slightly discolored owing to exposure to light during the washing process, but a deep discoloration is due to the presence of occluded silver nitrate and indicates insufficient washing.)

PROCEDURE FOR VOLHARD METHOD.

Filter the ash solution into a 200 cc. glass-stoppered volumetric flask. Wash the filter, dilute, and add a measured excess of standard silver nitrate solution of 0.1 *N* strength or weaker. Stopper the flask and shake until the silver chloride becomes granular, make up to the mark with cold distilled water, mix, and allow to stand in the dark until the precipitate settles clear. Filter the entire solution through a dry filter paper, pipet 100 cc. of the filtrate into a clean flask, and titrate back with standard 0.1 *N* thiocyanate solution and ferric alum indicator. Calculate the chlorine from the volume of the standard silver nitrate solution consumed.

SUMMARY.

1. Methods for the determination of chlorine in plant material that involve direct incineration were found to be as inaccurate and unreliable as other authorities have maintained.

2. In direct ashing methods carbon compounds, as well as sulfur and phosphorus, cause loss of chlorine owing to volatilization.

3. The possibilities of a direct method for determining chlorine without incineration in highly colored or possibly colloidal plant material is not promising.

4. Ashing plant material in the presence of a chlorine fixative appears to be the only practical method available to analysts.

5. Sodium carbonate as a chlorine fixative is feasible and satisfactory provided it is present in sufficient quantity. The minimum proportion of sodium carbonate to chlorine for the purpose of fixing chlorine during ashing is 5 to 1.

6. The present official gravimetric method for the determination of chlorine does not always give consistent results, possibly owing to occlusions and solubility factors.

7. A modification of the gravimetric method based on the use of dilute silver nitrate, precipitating the chlorine in the cold, and violently agitating for the purpose of producing an insoluble granular precipitate that can be thoroughly cleaned by a large quantity of cold water wash solution, gave satisfactory results.

8. The modified gravimetric, Gay-Lussac, and Volhard methods were found to be equally reliable and accurate for determining chlorine in ashed plant material in quantities ranging from 3 to over 100 mg. After the plant material has been prepared for the chlorine determination the analyst may take his choice.

The associate referee wishes to acknowledge the advice and assistance of H. J. Wichmann of the San Francisco Station of the Food, Drug and Insecticide Administration in the planning of the work and the preparation of this report.

RECOMMENDATIONS¹.

It is recommended—

(1) That collaborative work be undertaken on a method for the determination of inorganic chlorine in plant material.

(2) That future work include investigations to ascertain whether or not organic chlorine can be determined by ashing methods.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1928, 11: 71.

CONTRIBUTED PAPERS.

EFFECT OF SILICA DISHES IN THE DETERMINATION OF POTASH.

By L. D. HAIGH¹ (Agricultural Experiment Station, Columbia, Mo.).

At the meeting of this association in October, 1926, the writer presented data² showing variations in the results obtained in the determination of potash by the official method. These variations were attributed to the presence of water-soluble phosphates in solution. As a remedy there was proposed a modification of the preliminary washing, as followed in the official method, whereby the phosphates are removed before ignition of the residue.

The results by the two methods presented at that time were usually lower by the regular official method than by the modified method, although in a few cases there was apparent agreement. Since the publication of the former paper, other chemists have compared the official method and the proposed modified method. Some reported that the two methods gave practically the same results on the same sample and that they did not observe any tendency of the regular official method to give lower results. After further study and observation it was concluded that this disagreement might be attributed to the kind of dishes used for the ignition of the potash residue. In obtaining the results reported last year, the writer used silica dishes, while the chemists who failed to obtain consistent differences in results by the two methods used platinum dishes.

A. P. Kerr also studied the causes of variations in the results obtained by the potash method, and in correspondence with the writer suggested that the ignition in silica dishes, when phosphates are present, has a tendency to cause low results when the regular official method is used.

The effect of ignition of the residue in silica and platinum dishes may be observed by comparing the accompanying figures. Data on three fertilizer mixtures analyzed by the two methods are given. Sample C, which consisted of a simple mixture of acid phosphate and potassium chloride, gave the most divergent results with the official method when silica dishes were used. It appears that if water-soluble phosphates are present when the potash residues are ignited in silica dishes, greater variations in the results are to be expected than when the ignition is made in platinum dishes. When the phosphates are removed, the variation between the results obtained when platinum and silica dishes are used is minimized. It seems quite probable that the phosphates—or free phosphoric acid formed—attack the silica dishes and render some of the potash insoluble in the process.

¹ Presented by C. H. Jones.

² *This Journal*, 1927, 10: 220.

Potash results.

OFFICIAL METHOD.

FERTILIZER	IGNITED IN SILICA		IGNITED IN PLATINUM	
	<i>per cent</i>	<i>average per cent</i>	<i>per cent</i>	<i>average per cent</i>
Sample A	3.07	3.12	3.22	3.16
	3.03		3.13	
	3.14		3.14	
	3.24		3.13	
Sample B	9.04	9.02	9.37	9.31
	9.04		9.35	
	9.02		9.26	
	8.97		9.24	
Sample C	3.56	3.73	5.93	5.84
	3.90		5.83	
	3.96		5.71	
	3.49		5.90	

MODIFIED METHOD.

Sample A	2.60	2.74	2.99	2.93
	2.77		2.97	
	2.85		2.87	
	2.72		2.90	
Sample B	9.18	9.10	9.04	9.10
	9.04		9.22	
	9.01		8.93	
	9.15		9.21	
Sample C	5.98	5.81	6.08	5.97
	5.90		5.92	
	5.82		5.87	
	5.74		6.01	
	5.61		5.99	

WHAT CONSTITUTES AN ADEQUATE SAMPLE?

By J. C. MUNCH and G. L. BIDWELL (Food, Drug and Insecticide Administration, Washington, D. C.).

A sample is a portion of a given lot of material that is so taken or selected that it corresponds in certain specified particulars with the entire lot. The proportion of the original material necessary to make the sample "adequate" depends on a number of different considerations. These include the homogeneity or degree of heterogeneity of the material, the degree of accuracy of the measurements or analytical methods to be employed, and the use that will be made of the results when obtained. It is obvious that if the material under consideration is completely and uniformly homogeneous a very small portion taken at random from any place in the lot will constitute an adequate sample;

that is, its analysis will give the true composition of the original material with as great accuracy as can be obtained with the analytical methods used. At least no error due to the sampling procedure will be introduced into the analytical results. This ideal condition seldom if ever obtains.

As the degree of heterogeneity of the material increases, the difficulty of obtaining an adequate sample increases. A little reflection will show that too small a portion from a given lot of material will not truly correspond in composition or properties to the original material. On the other hand, too large a portion, while more truly representative, entails much labor in collection and analysis. The happy medium, from the practical standpoint, is a sample so small as to minimize the cost of collection and analysis, but large enough to be sufficiently representative for the purpose in view. The determination of the degree of accuracy needed is an administrative question pure and simple. It must be determined after a common-sense consideration of the cost and character of sample, purpose of the examination, etc.

Another important question that arises after it has been decided what size of sample is required from a given lot of material, is how large a sample must be taken from a lot of a different size in order to get equally accurate results.

Careful mathematical study has shown that adequate samples should consist of such a number of individuals, as is proportional to the square root of the number of individuals in the different lots. For the general run of material, such as flour and feeds, the square root gives an adequate sample. For material showing a greater degree of heterogeneity than usual, a multiple of the square root is needed and for material showing less variability a submultiple of the square root will suffice.

The derivation of the square-root rule, which has been used successfully in the sampling of flour, feed, etc., is an outgrowth from the formula

for the probable error of a correlation coefficient, $P. E. = 0.6745 \sqrt{\frac{1 - r^2}{N}}$.

$P. E.$ means the probable error and r is the correlation coefficient. As the allowable r and $P. E.$ are established by frequent assays and in accordance with administrative decision, they may be considered as constants representing and defining the degree of accuracy desired. Therefore the expression becomes: Accuracy of sampling varies as the square root of N .

A formula may now be selected for the number of individual samples to be collected from the whole lot to form a representative and adequate sample.

Let S represent the number of units in the sample and let N represent the number in the whole lot. Then if c is the factor to represent the degree of accuracy required, the formula reads:

$$S = c \sqrt{N}.$$

To put it in plain language, if 25 sacks of a lot of 625 sacks of feed are probed and the portions taken are mixed for a sample, and an adequate sample is thus obtained, then from a lot containing 50 sacks, seven should be probed to get a sample of an equal but no greater degree of accuracy.

DETERMINATION OF HIGH-BOILING PHENOLS IN A COAL-TAR CREOSOTE—CASTOR OIL SOAP DISINFECTANT.

By J. N. TAYLOR (Insecticide, Fungicide, and Caustic Poison Control, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C.).

The presence of high-boiling phenols in samples of commercial cresylic acid has been noted recently by Chapin¹. The growing tendency to utilize these high-boiling phenols in the manufacture of so-called saponified cresol solutions and in disinfectants which also contain hydrocarbon oils is probably due to the fact that the Tariff Act of 1922 permits a low rate of duty upon tar-acids which upon being subjected to distillation yield in the portion distilling below 215°C. a quantity of tar acids equal to or more than 75 per cent of the original distillate. Such acids are now imported in large quantities and are much cheaper than cresol. Because of the low volatility of high-boiling phenolic bodies, their quantitative determination by the method requiring steam distillation is somewhat difficult.

COMMERCIAL SOURCES OF HIGH-BOILING PHENOLS.

For many years high-boiling phenolic bodies have been obtained from the tar produced by cooling the waste gases from blast furnaces or that produced during the thermal decomposition of coal in vertical retorts. Of late the tar produced during the low-temperature carbonization of coal has become another source of high-boiling phenolic bodies, and, according to Caracristi² and to Morgan and Soule³, it is being produced in increasingly large quantities. Some of these phenols find their way into commercial articles used in disinfection, markedly increasing their bactericidal efficacy.

COAL TAR CREOSOTE DISINFECTANTS.

Disinfectants prepared from coal-tar creosotes containing high-boiling phenols have been produced in England for some time⁴. Burke and Caplan⁵, in this country, have recently called attention to disinfectants pre-

¹ U. S. Dept. Agr. Bull. 1308, 1924.

² *Chem. Age*, 1923, 31: 361.

³ *Chem. Met. Eng.*, 1922, 26: 923, 977; *Ind. Eng. Chem.*, 1923, 15: 587, 693.

⁴ *Lancet* (London), 1909, 11: 1454, 1516; *Ind. Chemist* (London), 1926, 11: 89.

⁵ *Ind. Eng. Chem.*, 1927, 19: 34.

pared from creosotes obtained from low-temperature coal tar, with special reference to the pink color that develops upon dilution with water. This is attributed to the presence of a mixture of orthodihydric phenols, including homologs of catechol. These compounds may be removed from the creosote before incorporation with a soap and water, although the coloration that they produce does not necessarily detract from the value of the article. The odor of low-temperature coal-tar creosote disinfectants, upon dilution with warm water, is distinctive, less pleasant than the odor of disinfectants manufactured from coke-oven creosotes, and somewhat more empyreumatic. The viscosity of undiluted low-temperature creosote disinfectants is greater than that of disinfectants manufactured from high-temperature creosote, while their specific gravity is lower.

As high-boiling phenols are difficultly volatile with steam, the steam-distillation method does not afford adequate means for their quantitative isolation and subsequent determination. Weiss¹ makes use of direct distillation in determining tar acids in tar oils. In the isolation of high-boiling phenols from so-called saponified cresol solutions, Chapin successfully employs direct distillation.

Preliminary to an inquiry into the applicability of this procedure to coal-tar creosote disinfectants containing high-boiling phenols incorporated with various soaps, one containing castor oil soap was examined.

EXPERIMENTAL.

The tar acid used in the experiments here described was obtained from a corporation engaged in the complete gasification of coal and recovery of the by-product low-temperature tar and was submitted to fractional distillation. The portion boiling up to 280°C. was refractionated, 89 per cent distilling between 205° and 225°C. and 11 per cent between 225° and 280°C., the indication being that the phenols consisted mainly of xlenols with a smaller quantity of higher boiling phenols. It had a specific gravity of 1.0317 at 25°/4°C. and was incorporated with hydrocarbon oils, castor oil soap and water in the proportions stated later. The hydrocarbon oils consisted of the neutral oils extracted from a high-temperature creosote and yielded no diethyl sulfate residue². The soap was prepared by saponifying castor oil with caustic potash solution, a slight excess of alkali being present in the finished product. The sample of castor oil employed had a saponification number of 180.

PREPARATION OF DISINFECTANT.

Test-tube experiments demonstrated that a stable homogeneous mixture, capable of forming a stable emulsion upon dilution, must contain

¹ *Ind. Eng. Chem.*, 1918, 10: 911.

² *Ibid.*, 1927, 19: 76; *Chem. News* (London), 1927, 134: 212.

the phenols, hydrocarbon oils, soap, and water in proper proportion. The proportion of phenols desired in the product influences the relative quantities of hydrocarbon oils, soap and water that can be incorporated. It was also found that the oils had to be added slowly to the phenol-soap-water mixture at a low temperature and with constant stirring in order to effect complete miscibility. Fair results were obtained by adding a soap-water-hydrocarbon oil mixture to the phenols. Following these preliminary tests a disinfectant was made having the composition noted below:

	<i>per cent by weight</i>
Phenols.....	25
Hydrocarbon oils.....	32
Castor oil soap.....	19
Water.....	24

The specific gravity of the product was 1.0568 at 25°/4°C.

DETERMINATION OF TOTAL PHENOLS.

Fifty grams of the disinfectant thus prepared was subjected to steam distillation until one liter of distillate had been collected. The phenols were recovered and measured according to the procedure prescribed by Chapin¹ for the determination of phenols in coal-tar creosote dips. Duplicate determinations yielded 23.2 and 23.5 per cent, respectively, of phenols.

The technic employed in determining the phenolic bodies by direct distillation was also substantially that prescribed by Chapin in Bulletin 1308 for "saponified cresol solutions". Fifty grams of disinfectant was weighed into a 250 cc. Pyrex side-neck distilling flask, 2.5 grams of sodium bicarbonate and 0.5 gram of magnesium carbonate were added, and the sides of the neck were washed down with 50 cc. of a high-boiling refined petroleum distillate that had been previously washed with alkali. The flask was connected with a Liebig condenser, and the distillate was collected in a Weiss, type 2, measuring tube. Distillation was conducted over a free flame at a slow rate until the distillate attained a deep yellow color. The water that collected in the tube was transferred to a separatory funnel, and any phenols present were salted out, taken up in kerosene, and added to the distillate in the measuring tube. After washing with 10 cc. of 10 per cent sulfuric acid and discarding the acid wash, the volume in the tube was noted at 25°C. The phenolic bodies were removed with successive 80 and 60 cc. portions of 10 per cent sodium hydroxide solution, and, after thorough draining, the final volume in the tube was again noted at the same temperature.

Duplicate determinations gave diminutions in volume of 11.95 cc. and 12.03 cc., respectively. These corresponded to 24.4 grams and 24.8 grams

¹ U. S. Dept. Agr. Bur. Animal Industry Bull. 107, 1908.

of phenols per 100 grams of disinfectant, respectively. Calculated upon a volume basis, 100 grams of the disinfectant yielded 25.3 cc. and 25.4 cc., respectively (theoretical, 25.6 cc. of phenols per 100 cc. of disinfectant).

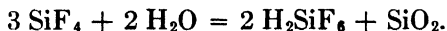
CONCLUSION.

High-boiling phenols in an emulsion-producing type of disinfectant containing a castor-oil soap may be more satisfactorily and accurately determined by distilling directly than by employing steam distillation.

VOLATILIZATION METHOD FOR THE DETERMINATION OF FLUORINE WITH SPECIAL REFERENCE TO THE ANALYSIS OF PHOSPHATE ROCK.

By D. S. REYNOLDS, W. H. ROSS, and K. D. JACOB (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.).

Numerous methods have been proposed for the determination of fluorine, but with the exception of the volatilization method all of them become impractical in the presence of high percentages of phosphates. This method is based on the formation and volatilization of silicon tetrafluoride when a material containing fluorine is mixed with silica and decomposed with concentrated sulfuric acid. According to different authorities the silicon tetrafluoride may be (1) collected and measured as such; (2) passed into water or aqueous solutions of certain alkali salts and the fluorine calculated from the weight of silica or alkali silicofluoride precipitated; or (3) passed into water and the fluorine determined by titration of the hydrofluosilicic acid formed as represented by the equation



The titration method proposed by Offermann¹ was investigated by Wagner and Ross², who proposed a number of improvements in the method with particular reference to the purification of the evolved silicon tetrafluoride. Their results show that the method is more convenient and rapid than the other modifications mentioned; that it is applicable to the determination of fluorine over a wide percentage range; and that it gives equally as good results in the presence of phosphates as in the analysis of pure materials.

The Wagner-Ross modification, accordingly, was selected for a survey that was recently undertaken in this laboratory of the fluorine occurring in phosphate rock secured from widely separated sources. As a preliminary step in this work, analyses were made of two standard fluorine

¹ *Z. angew. Chem.*, 1890, 3: 615.

² *J. Ind. Eng. Chem.*, 1917, 9: 1116.

samples of known purity for the purpose of testing the accuracy of the method. The standard used in one of these tests was a specially prepared sample of sodium fluoride that had been repeatedly crystallized in a large platinum dish. The second standard was a sample of carefully selected fluorite¹, CaF_2 , which was ground to 200 mesh in an agate mortar. A portion of this sample was treated with concentrated sulfuric acid and ignited to $400^\circ\text{--}500^\circ\text{C.}$, the treatment being repeated to constant weight. The average of several closely agreeing determinations gave 72.21 per cent calcium oxide as calculated directly from the weight of calcium sulfate obtained. The calcium sulfate residues were then dissolved in hydrochloric acid, and the calcium was precipitated as calcium oxalate and weighed directly as calcium oxide. The average result was 71.53 per cent calcium oxide, based on the weight of calcium fluoride taken. Since pure calcium fluoride theoretically contains 71.82 per cent calcium oxide, the results obtained indicate a purity of 99.60 per cent for this particular sample of fluorite.

The fluorine content of the standard samples was then carefully determined by the method described by Wagner and Ross. The method was followed carefully in every detail, but the fluorine found in repeated determinations did not exceed 85 per cent of the total present. Qualitative tests indicated that the low results were due to retention of some of the fluorine in the digestion flask and not to decomposition of the silicon tetrafluoride in other parts of the apparatus. An attempt was accordingly made to bring about a more complete volatilization of the fluorine. The standard sample of fluorite was used in preference to sodium fluoride because of the probable similarity between the physical and chemical properties of fluorite and those of the fluorine compounds occurring in phosphate rock.

The results obtained in this work, together with a description of the method and apparatus finally adopted, are given in this paper.

EXPERIMENTAL.

In the method and apparatus described by Wagner and Ross the silicon tetrafluoride volatilized by boiling the mixture of sample and silica with sulfuric acid is carried through the purification apparatus to the absorption tube by means of a current of carbon dioxide. Such a procedure requires constant attention and is a source of considerable danger to the operator in case the digestion flask should break. Besides, any leak in the apparatus is accompanied by loss of fluorine. It was found that the use of dry air instead of carbon dioxide did not affect the results and that by drawing a stream of the former through the apparatus all danger of loss of fluorine by leakage is avoided.

¹ This sample was furnished by E. V. Shannon, Mineral Division, U. S. National Museum.

Many different kinds of digestion flasks were designed and tested out in the apparatus with the view to securing more complete volatilization of the fluorine. The flask finally adopted (Fig. 2) is so constructed that boiling of the acid to dispel the fluorine is unnecessary. Heating the flask and its contents in a small home-made or standard-type electric furnace to a temperature below the boiling point of the acid and aerating with a stream of dry air drawn through the apparatus is a simpler, safer, and more effective procedure.

EFFECT OF TEMPERATURE AND STRENGTH OF SULFURIC ACID.

Experiments were made to determine the optimum temperature of digestion and the concentration of sulfuric acid required for maximum recovery of fluorine from the standard sample of fluorite ground to a fineness of 200 mesh. The general procedure followed was the same as that given later under the description of the method and apparatus. Temperatures were determined by means of a thermometer placed against the wall of the digestion flask at a point about halfway up the sulfuric acid column. In each case the temperature was raised to the desired point in about 15 minutes and maintained constant at this point for 45 minutes. The concentration of the sulfuric acid (H_2SO_4) used in these experiments was varied from 95.1 to 99.1 per cent, as determined by titration with standard alkali.

TABLE 1.

Effect of temperature of digestion and concentration of sulfuric acid on recovery of fluorine from calcium fluoride.

(0.05 gram calcium fluoride \approx 0.0242 gram fluorine.)

CONCENTRATION OF ACID (H_2SO_4)	TEMPERATURE	FLUORINE RECOVERED	
		grams	per cent
<i>per cent by weight</i>	<i>°C.</i>		
95.1	200-250*	0.0225	93.0
95.1	200-250*	0.0228	94.2
95.1	300	0.0196	81.0
95.1	300	0.0203	83.9
96.6	300	0.0226	93.4
96.6	300	0.0219	90.5
97.0	250-300*	0.0221	91.3
97.0	300	0.0218	90.1
97.0	300	0.0223	92.1
97.5	300	0.0222	91.7
97.5	300	0.0224	92.6
98.3	240-260	0.0226	93.4
98.3	240-260	0.0228	94.2
98.3	300	0.0224	92.6
98.3	300	0.0225	93.0
98.3	300	0.0228	94.2
98.3	300	0.0227	93.8
98.3	340-350	0.0202	83.5
98.3	340-350	0.0215	88.8
98.3	340-350	0.0211	87.2
99.1	300	0.0139	57.4
99.1	300	0.0138	57.0

* Temperature was rapidly raised to the minimum indicated and then slowly increased to the maximum.

The 200 mesh quartz used as a source of silica in the reaction mixture originally contained 0.058 per cent of fluorine. It was rendered practically fluorine-free by digesting with 98 per cent sulfuric acid at 250°–300°C. for about 2 hours, and the purified material was used in all the determinations. All results are corrected for sulfites and sulfates found in the hydrofluosilicic acid solution after titration with standard alkali. This correction was equivalent to 0.05–0.15 cc. of 0.1 *N* sodium hydroxide when the material analyzed was practically free from organic matter.

The data given in Table 1 indicate that recovery of fluorine as complete as the maximum obtained with more concentrated acids can be obtained by digesting the sample with 95.1 per cent sulfuric acid when the temperature of the reaction mixture is rapidly raised to 200°C. and then slowly increased to 250°C. in the course of about 45 minutes. On the other hand, when the temperature was rapidly raised to 300°C. the percentage of fluorine recovered was considerably lower. This variation may be explained by the assumption that at the lower temperatures no appreciable increase occurs in the concentration of the 95.1 per cent acid during the course of the digestion, and that consequently the gases and vapors present in the reaction tube do not contain sufficient moisture to cause appreciable reaction with the silicon tetrafluoride that is evolved. As the temperature is raised to 300°C. the quantity of moisture in the reaction gases and vapors increases, and a certain amount of the silicon tetrafluoride is decomposed, probably before it reaches the first sulfuric acid tube in the purification train.

With acids varying in concentration from 96.6 to 97.5 per cent, the recovery of fluorine varied irregularly between 90.1 and 93.4 per cent of the total present when the digestions were carried out at 300°C. With 98.3 per cent sulfuric acid the recovery varied irregularly from 92.6 to 94.2 per cent when the digestions were made at temperatures varying from 240°–260° to 300°C., but at boiling temperatures, 340°–350°C., the recovery of fluorine decreased to 83.5–88.8 per cent. Increasing the concentration of the acid to 99.1 per cent resulted in a recovery of only about 57 per cent of the fluorine when the sample was digested 1 hour at 300°C. This was probably due to the slight ionization of the highly concentrated acid even at high temperatures and the consequent decrease in the rate of reaction.

The data in general indicate that in the analysis of pure compounds as good results are obtained by digesting the sample with about 95 per cent sulfuric acid at temperatures of 200°–250°C. as by digesting with 98–98.5 per cent acid at 300°C. However, in the analysis of phosphate rock it was found that the highest and most consistent results were obtained by digesting the sample with 98–98.5 per cent sulfuric acid at 300°C. When lower strength acids were used, the results were frequently inconsistent.

EFFECT OF SIZE AND FINENESS OF SAMPLE.

Analyses of the standard sample of fluorite indicated that when the digestions were made with 98.3 per cent sulfuric acid at 300°C. recovery of the fluorine was not appreciably increased by grinding the sample finer than about 80 mesh. However, in the analysis of phosphate rock, higher and more uniform results were always obtained on samples ground to a fineness of about 200 mesh.

Comparative analyses of different quantities of 200-mesh fluorite were made to determine what effect size of sample has on the total fluorine recovered. The data given in Tables 2 and 3 indicate that approximately the same percentage recovery is obtained when the quantity of fluorine varies from 0.0121 to 0.0484 gram, but that with larger quantities of fluorine the percentage recovery appears to decrease slightly.

TABLE 2.

Recovery of fluorine from different quantities of calcium fluoride.

SAMPLE TAKEN		FLUORINE RECOVERED	
CaF ₂	Fluorine		
<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>per cent</i>
0.050	0.0242	0.0226	93.4
0.050	0.0242	0.0228	94.2
0.075	0.0363	0.0333	91.7
0.075	0.0363	0.0339	93.4
0.100	0.0484	0.0449	92.8
0.100	0.0484	0.0455	94.0
0.150	0.0726	0.0662	91.2
0.150	0.0726	0.0668	92.0

EFFECT OF IMPURITIES IN SAMPLE ON RECOVERY OF FLUORINE.

Wagner and Ross have shown that the presence of tricalcium phosphate does not interfere in the recovery of fluorine from sodium fluoride and sodium fluosilicate. This observation was confirmed by the writers in the analysis of a mixture containing 1 gram of calcium fluoride and 9 grams of calcium phosphate. This mixture was prepared from 200-mesh materials, and uniform composition was obtained by prolonged agitation in a bottle with a number of small rubber-coated lead pellets. Mixtures of calcium fluoride with ferric phosphate, aluminum phosphate, anhydrous borax, and sodium arsenate were also analyzed. In all cases, the digestions were made with 98.3 per cent sulfuric acid at 300°C.

The results in Table 3 show that the presence of iron, aluminum, sodium, phosphorus as phosphate, and arsenic as arsenate has no effect on the determination, the recovery of fluorine being as complete in the presence of these materials as with pure calcium fluoride alone. On the other hand, the presence of boron in the form of borax has a very detri-

TABLE 3.

Effect of impurities in sample on the recovery of fluorine from calcium fluoride.

SAMPLE USED	FLUORINE		FLUORINE RECOVERED
	Taken	Found	
	<i>gram</i>	<i>gram</i>	<i>per cent</i>
$\text{CaF}_2 + \text{Ca}_3(\text{PO}_4)_2$	0.0121	0.01125	93.0
	0.0121	0.01115	92.1
	0.0242	0.0226	93.4
	0.0484	0.0450	93.0
	0.0242	0.0226	93.4
0.05 gram CaF_2 + 0.1 gram FePO_4	0.0242	0.0223	92.1
0.05 gram CaF_2 + 0.1 gram AlPO_4	0.0242	0.0225	93.0
0.05 gram CaF_2 + 0.1 gram Na_2AsO_4	0.0242	0.00381	15.7
0.05 gram CaF_2 + 0.5 gram $\text{Na}_2\text{B}_4\text{O}_7$	0.0242	0.0128	52.9

mental effect, the percentage of fluorine recovered varying from 15.7 in the presence of 0.5 gram of borax to 52.9 with 0.1 gram.

The detrimental effect of boron is probably due to the formation of volatile boron trifluoride, which is appreciably soluble in concentrated sulfuric acid. According to Mellor¹, when silicon tetrafluoride is passed through vitreous boric oxide it is converted largely into boron trifluoride. From this it is evident that selective reaction occurred between the boron and the hydrofluoric acid liberated in the digestion flask even in the presence of a large excess of silica. While the results obtained on the effect of boron have no particular relation to the analysis of phosphate rock, they nevertheless show that the method should not be used indiscriminately for the determination of fluorine in samples of unknown composition. The effect of other impurities, such as chromium, vanadium, etc., that may occur in phosphate rock was not determined, but they are usually present in such small quantities that they probably would not interfere to any appreciable extent, if at all.

EFFECT OF HIGH TEMPERATURES ON THE FLUORINE CONTENT OF PHOSPHATE ROCK.

If the sample of phosphate rock to be analyzed contains considerable organic matter, as is frequently the case, it is desirable to remove at least a large portion of it before making the determination. This is done by ignition at as low a temperature as possible. Experiments were carried out to determine the amount of fluorine lost on ignition at different temperatures and also whether the addition of a small quantity of lime would prevent this loss. The Bureau of Standards' standard sample No. 56, Tennessee brown rock phosphate, was used in all these experiments. The material was ground to 200 mesh and dried to constant weight at 105°C. In all of the determinations 1 gram of phosphate

¹ A Comprehensive Treatise on Inorganic and Theoretical Chemistry, vol. V, p. 121. 1924.

rock, intimately mixed with 0.5 gram of 200-mesh quartz, was digested with 98.3 per cent sulfuric acid at 300°C. for 1 hour. The results are corrected for sulfites and sulfates found in the solution after titration.

TABLE 4.

Effect of high temperatures on the fluorine content of Tennessee brown rock phosphate.

TEMPERATURE	PERIOD OF HEATING	CaO ADDED	FLUORINE FOUND	
°C.	hours	gram	gram	per cent
....	0.0335	3.35
....	0.0334	3.34
600	3	0.0324	3.24
600	3	0.25	0.0330	3.30
500-600	2	0.50	0.0331	3.31
500-600	2	0.50	0.0333	3.33
750-800	2	0.0283	2.83
750-800	2	0.0291	2.91
750-800	2	0.25	0.0329	3.29
750-800	2	0.25	0.0323	3.23
750	1.5	0.50	0.0333	3.33
750	1.5	0.50	0.0335	3.35
900	1	0.25	0.0310	3.10
900	1	0.25	0.0313	3.13
900-925	1	0.50	0.0257	2.57

The data given in Table 4 show that considerable fluorine was lost when samples of phosphate rock containing no admixed lime were ignited at 750°-800°C. for 2 hours. Loss was prevented at this temperature by mixing the sample with 0.5 gram of lime prior to ignition, but addition of lime did not prevent loss of fluorine when the samples were ignited at 900°-925°C. Satisfactory removal of organic matter without serious loss of fluorine was effected by mixing 1 gram of phosphate rock with 0.5 gram of lime and igniting in a muffle furnace at 500°-600°C. for 2 hours. This procedure gave good results in the analysis of approximately 100 samples of various grades and types of phosphate rock from widely separated deposits.

DETAILS OF ANALYTICAL METHOD.

REAGENTS.

(1) *98.5 per cent sulfuric acid.*—This is most conveniently prepared by adding sufficient fuming sulfuric acid to ordinary concentrated sulfuric acid to give a solution containing about 99 per cent sulfuric acid. The solution is heated in a large beaker, preferably on a hot plate, for about 1 hour after it begins to fume strongly, in order to remove free sulfur trioxide and sulfur dioxide. The latter appears to be present in appreciable quantity in some samples of sulfuric acid prepared in the above manner. If the final product contains more than 98.5 per cent sulfuric acid, it is diluted with the correct quantity of 95 per cent acid, or if it contains less than 98 per cent sulfuric acid, more fuming acid is added. In either case, the acid as finally used should contain 98-98.5 per cent sulfuric acid, as determined by titration with standard alkali.

(2) *Silver sulfate.*—A 10 per cent solution of silver sulfate in 98-98.5 per cent sulfuric acid is prepared and heated until it fumes strongly in order to remove volatile impurities.

Certain samples of supposedly C. P. silver sulfate have been found to contain considerable nitrate. This solution is used to absorb the hydrogen chloride that is evolved when chlorides are present in the sample.

(3) *Chromium trioxide*.—The pure material is dried at 105°–110°C. and finely ground. A suspension, containing preferably a large excess of solid chromium trioxide, is made in 98–98.5 per cent sulfuric acid. This reagent is used to absorb sulfur dioxide, nitric acid, and oxides of nitrogen.

(4) *Silica*.—The commercial grade of quartz flour is satisfactory. It should be ground to 200 mesh and ignited at a low red heat to remove organic matter and moisture. Samples of quartz flour have been found to contain as high as 0.06 per cent of fluorine. Any fluorine present can be removed by digesting the material with sulfuric acid, preferably about 98 per cent strength, at temperatures of 250°–300°C.

(5) *Standard alkali and acid*.—Tenth-normal solutions of sodium hydroxide and hydrochloric acid are prepared. One cubic centimeter of 0.1 *N* sodium hydroxide is equivalent to 0.0019 gram of fluorine.

APPARATUS.

The apparatus required for the determination is assembled as shown in Fig. 1 and is composed of the following individual pieces:

(1) A gas-washing bottle, *A*, containing concentrated sulfuric acid for removal of moisture from the inlet air.

(2) A cylinder, *B*, loosely packed with glass wool and phosphorus pentoxide, also for moisture removal.

(3) A reaction flask, *C*, for digesting the sample with 98–98.5 per cent sulfuric acid. The flask, shown in detail in Fig. 2, is made of Pyrex glass and is designed especially for this purpose. It is composed of a principal tube, *c*, having an opening, *d*, for introducing the sample and acid. Dry air is drawn into the tube through *a* and the gaseous reaction products pass out through the bulbous tube, *e*. The porcelain plate, *b*, about 2.5 cm. in diameter and having perforations about 0.3 mm. in diameter, makes close contact with the walls of the tube, *c*, and is held in place by indentations in the glass above and below the plate. It serves to prevent portions of the sample from being carried up into the air-inlet tube, *a*, when the acid is added, and at the same time it permits free passage of air through the acid during digestion.

(4) A furnace, *D*, with rheostat, *E* (not shown), for heating the reaction tube. A type 84, multiple unit, electric crucible furnace is satisfactory and convenient for this purpose. A suitable electric furnace may be made by winding the proper quantity of nickel-chromium wire on a porcelain, alundum, or fire-clay core of the proper size. The reaction flask should be placed in the furnace so that the level of the sulfuric acid is below the top of the furnace, which is covered with a piece of asbestos board containing openings for the tube and thermometer.

(5) A Bowen potash tube, *F*, containing 98–98.5 per cent sulfuric acid.

(6) A Schmitz sulfuric acid tube, *G*, containing the 10 per cent solution of silver sulfate in 98–98.5 per cent sulfuric acid. The cylindrical arm of this tube is filled with glass beads moistened with the silver sulfate solution.

(7) Two Bowen potash tubes, *H*, having the exit end of the first tube fused to the inlet end of the second. Both tubes contain the suspension of chromium trioxide in 98–98.5 per cent sulfuric acid. The contents of the tubes are renewed when most of the chromium trioxide has been removed from suspension.

(8) A glass tube, *I*, filled with glass wool. This tube is approximately 0.5 cm. in diameter and is bent at right angles about 16 cm. from the inlet end. Wagner and Ross have pointed out that it is necessary to pass the reaction gases through a tube filled with glass wool in order to remove sulfur trioxide efficiently. Care should be taken to

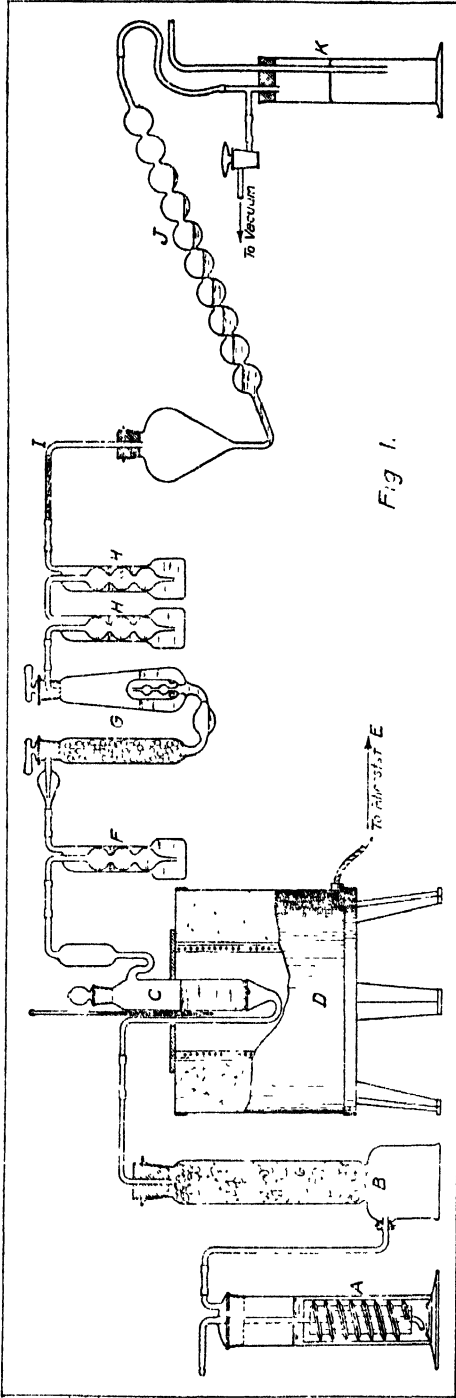


Fig. 1.

FIG. 1. APPARATUS FOR THE DETERMINATION OF FLUORINE IN PHOSPHATES.

replace the tube before the glass wool becomes completely saturated, as indicated by a perceptible coloration of the glass wool when it is heated to 100°–130°C.

(9) A Meyer sulfur tube, *J*, containing 50 cc. of distilled water and 10 cc. of 0.1 *N* hydrochloric acid and connected with the tube, *I*, by means of a rubber stopper. The exit end of the Meyer tube is connected with a source of vacuum through a pressure-regulating vessel, *K*, containing mercury.

All connections, except the one between the two Bowen tubes, *H*, are made with rubber, and the permanent rubber connections are coated with shellac. The ground-glass stopper in the reaction flask, *C*, is lubricated with 98–98.5 per cent sulfuric acid. The other ground-glass stoppers are lubricated either with sulfuric acid or a good grade of stopcock grease. When the apparatus is not in use, all openings are closed to prevent absorption of moisture from the air by the sulfuric acid solutions.

PROCEDURE.

The sample for analysis is ground to pass a 200-mesh sieve. Its weight will depend on the fluorine content of the material, but a maximum sample of 1 gram, equivalent to about 30–40 mg. of fluorine, is preferable in the analysis of phosphate rock. The sample must be thoroughly dry, and if it contains an appreciable quantity of organic matter, as is frequently the case with phosphate rock and other commercial materials, it should be intimately mixed in an agate mortar with about 0.5 gram of calcium oxide and ignited in a muffle furnace at 500°–600°C. for 2 hours.

The prepared sample is thoroughly mixed with 200-mesh silica in an agate mortar and transferred to the dry digestion flask. Addition of anhydrous copper sulfate to prevent bumping, as recommended by Wagner and Ross, is not necessary when the tube is heated in an electric furnace and the reaction mixture is agitated by a current of air. If the sample does not exceed 0.5 gram, 0.5 gram of silica will be sufficient, but with larger samples a quantity of silica equivalent to the weight of the sample should be used. The digestion tube is connected in place in the furnace, and 40 cc. of 98–98.5 per cent sulfuric acid is added. If the sample contains considerable carbonate, the acid may be added slowly from a dropping funnel connected with the digestion flask through a rubber stopper, while a slow current of air is drawn through the apparatus. When all the acid has been added, the funnel and rubber stopper are quickly replaced by the ground-glass stopper. Most of the carbonate also may be removed, prior to digestion with sulfuric acid, by mixing the sample with about 0.5 gram of lime and igniting at 750°–800°C. for 1 hour, loss of fluorine being negligible in most cases.

After addition of the acid, air is drawn through the apparatus at such a rate that a practically continuous stream of bubbles is obtained. The temperature of the digestion tube is rapidly raised to about 250°C. and then slowly increased to about 300°C. in the course of about 45 minutes. The temperature is observed by means of a thermometer placed with the bulb against the wall of the digestion flask at a point about halfway up the sulfuric acid column. After the tube has been heated for about 15 minutes, it is shaken at frequent intervals to facilitate solution of the white scum that forms on the surface of the acid. The presence of fluorine is characterized by the formation of this white scum. Digestion of the sample, continued until the scum disappears, usually requires about 1 hour. Heating is then discontinued, and aeration is continued 15–20 minutes longer.

The Meyer sulfur tube is then disconnected, and its contents are transferred to a 500 cc. flask. The silica deposited in the tube is removed as completely as possible, and the tube rinsed several times with distilled water. The final volume of solution and rinsings in the flask is made up to 200–250 cc. Ten cubic centimeters of the standard hydrochloric acid is placed in another flask of the same size and diluted to the same volume. The contents of both flasks are heated to boiling under as nearly the same

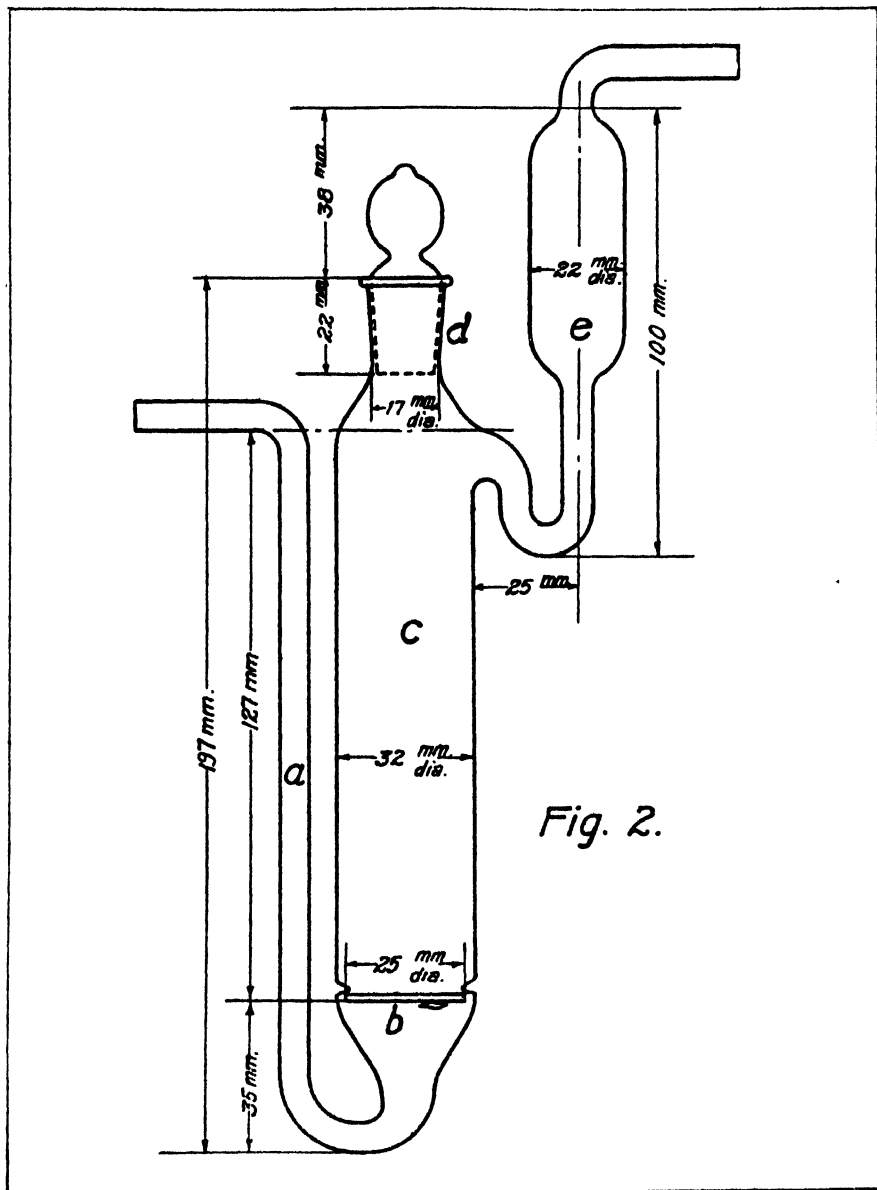


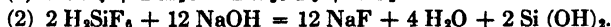
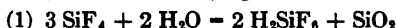
Fig. 2.

FIG. 2. REACTION FLASK USED IN THE DETERMINATION OF FLUORINE IN PHOSPHATES.

conditions as possible—an electric hot plate gives very uniform results—and the boiling is continued for 5 minutes to remove carbon dioxide and as much sulfur dioxide as possible. The contents of both flasks are titrated hot with 0.1 *N* sodium hydroxide, phenolphthalein being used as an indicator. The end point is usually fairly sharp, and it should be approached carefully.

The final results can be corrected for the small quantity of sulfite and sulfate that is usually present in the titrated solution with sufficient accuracy, in most cases, by adding bromine water to oxidize the sulfite to sulfate. The solution is allowed to stand overnight, and the milky solution obtained by adding 10 per cent barium chloride solution acidified with hydrochloric acid is compared with solutions containing 0.05–0.3 cc. of 0.1 *N* sulfuric acid in the same volume of water. The small quantity of silica present is usually in such a granular condition that it does not interfere with the test. The total sulfate found in samples containing but little organic matter should not exceed the equivalent of 0.2 cc. of 0.1 *N* sulfuric acid and is usually much less than this figure.

From the total sodium hydroxide titration there should be subtracted the equivalent of the fluorine blank on the reagents, the equivalent of the hydrochloric acid used in the absorption tube, and the equivalent of the sulfate found in the titrated solution to obtain the net sodium hydroxide equivalent of the fluorine content of the sample according to the following equations:



The hydrated silica does not react with the dilute standard alkali solution. The purpose of the hydrochloric acid in the Meyer tube is to decrease the tendency of sulfur dioxide to oxidize to sulfur trioxide, but it is questionable whether there is any advantage in its use if the quantity of organic matter in the sample is small. Dilute solutions of hydrofluosilicic acid do not lose fluorine when boiled for a short time.

The procedure outlined has been used for the determination of fluorine in approximately 100 samples of different grades and types of phosphate rock from domestic and foreign deposits. No difficulty was experienced in obtaining duplicate results that agreed within 0.1 per cent and usually to within less than 0.05 per cent of fluorine. The results of this investigation will be given in the following paper.

SUMMARY.

(1) A study has been made of the volatilization method for the determination of fluorine, with special reference to the analysis of phosphate rock.

(2) A greatly improved flask for digesting the sample with silica and sulfuric acid has been developed.

(3) Recovery of 92–94 per cent of the fluorine present in pure calcium fluoride was obtained by digesting the sample with 98–98.5 per cent sulfuric acid for 1 hour at 300°C.

(4) The presence of calcium, iron, and aluminum phosphates and sodium arsenate did not interfere in the recovery of fluorine from pure calcium fluoride, but boron in the form borax had a detrimental effect.

(5) Addition of lime prevented loss of fluorine when samples of phosphate rock were ignited at temperatures up to 750°C. in order to remove organic matter.

THE FLUORINE CONTENT OF PHOSPHATE ROCK.

By K. D. JACOB and D. S. REYNOLDS (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.).

Commercial phosphate rock almost invariably contains an appreciable quantity of fluorine, which is an important factor in the manufacture of such phosphatic fertilizers as superphosphate and double superphosphate. In the manufacturing process a portion of the fluorine is volatilized principally as silicon fluorides and, as such, it constitutes an industrial nuisance, the abatement of which is required by law in most localities. The volatilized silicon fluorides are usually absorbed in water, and the solution is allowed to run to waste. A few of the larger superphosphate plants have installed equipment for the recovery of fluorine from the reaction gases in which the gases are absorbed in water and the fluorine is precipitated as sodium fluosilicate by the addition of a solution of a sodium salt. However, this practice is by no means general, principally because of the comparatively small demand for fluorine compounds.

Recent experiments¹ (13), (14), (20) indicate that fluorides and fluosilicates possess valuable properties as agricultural poisons, particularly as insecticides and fungicides. It is likely that large quantities of these compounds will be used for this purpose when their value and specific properties become better known. Practically all superphosphate factories are located in agricultural districts, and the creation of a demand for fluorine compounds as agricultural poisons should result in their profitable recovery as by-products in the manufacture of superphosphate.

The writers have received numerous requests for information on the fluorine content of phosphate rock. In addition to its practical value, accurate data on this subject would undoubtedly be of some assistance in clearing up the question of the chemical constitution of phosphate rock. A search of the literature showed that the published figures on the fluorine content vary widely even on samples of the same grade of rock from the same general locality. In many cases these variations are undoubtedly due to the use of inaccurate analytical methods. After investigating the various methods that have been proposed for the determination of fluorine, Wagner and Ross (22) showed that with the exception of the volatilization method they are impractical in the presence of high percentages of phosphates. A further study of this method is reported in the preceding paper, "The Volatilization Method for the Determination of Fluorine With Special Reference to the Analysis of Phosphate Rock". An average recovery of about 93.5 per cent of the fluorine present in pure calcium fluoride was obtained, and the percentage recovery was not affected by the presence of large quantities of calcium, iron, and aluminum phosphates.

¹ Numerals in italics in parentheses refer to the bibliography at the end of the paper.

PURPOSE OF INVESTIGATION.

The present investigation was made to obtain accurate data on the fluorine content of the various commercial grades and types of phosphate rock from deposits in the United States. For comparative purposes, analyses were also made of several samples of phosphate rock from deposits in North Africa and from several of the more important phosphate islands. In a number of instances samples representing shipments of 30-5,000 tons were furnished by some of the phosphate mining companies and by manufacturing concerns that use large quantities of phosphate rock. In most cases, these samples were taken by means of automatic sampling devices and consequently were very desirable.

The method of analysis outlined in the preceding paper was followed closely. In all cases, except in the analysis of superphosphate and double superphosphate, the samples were ground to pass a 200-mesh sieve. One gram of the ground material was intimately mixed with 0.5 gram of freshly ignited calcium oxide and heated in a muffle furnace for 2 hours at 500°-600°C. in order to remove the larger portion of the organic matter that is always present in phosphate rock in varying quantities. The ignited sample was then intimately mixed with 1 gram of 200-mesh quartz, and the analysis was carried out as described. The results were corrected for a small blank on the reagents and for small quantities of sulfites and sulfates present in the titrated solution. Duplicate analyses were made on all samples, the results checking within 0.1 per cent of fluorine in all cases, and usually within less than 0.05 per cent. All the samples were analyzed for moisture by heating 2 grams of material for 1 hour at 105°-110°C., and the figures for fluorine were calculated on the dry basis. As previously pointed out the method used for the determination of fluorine accounts for about 93.5 per cent of the total present in the sample. All the figures for fluorine given in the tables are calculated to 100 per cent recovery on the basis of an actual recovery of 93.5 per cent of the fluorine in the moisture-free sample.

THE FLUORINE CONTENT OF FLORIDA PHOSPHATE ROCK.

At the present time the Florida phosphate deposits are the most important in the United States, the annual production of phosphate rock being greater than that in any other single district of the same size in the world. The two most important classes of phosphate in Florida at the present time are the hard-rock and land-pebble phosphates, the annual marketed tonnage of the latter being about twenty times that of the former. There are also large quantities of so-called "soft phosphates" in both the hard-rock and land-pebble phosphate districts. This "soft phosphate" is usually in a fine state of division and contains sufficient iron and aluminum to prevent its use in the manufacture of fertilizer by acid-treatment processes. It is generally closely associated

with the hard-rock and land-pebble phosphates and is usually lost during the process of concentrating the phosphate for market. Large individual deposits of "soft phosphate" are frequently found, but the commercial production of this material is small at the present time.

TABLE 1.
Fluorine content of Florida phosphate rock.

SAMPLE NO.	APPROXIMATE $\text{Ca}_3(\text{PO}_4)_2$ CONTENT	LOCATION OF DEPOSIT	FLUORINE
LAND-PEBBLE PHOSPHATE			
	<i>per cent</i>		<i>per cent</i>
618	67-68	Pierce	3.92
619	68	Nichols	3.98
567	68-70	Mulberry	3.86
436*	69	"	3.91
620	70	Nichols	3.92
628*	70-71	Bartow	3.97
568	70-72	Mulberry	3.97
437*	71	"	3.94
621	72	Nichols	3.96
569	72-73	Mulberry	3.92
438*	72.5	"	3.90
439*	74	"	3.87
570	74-75	"	3.95
622	74-75	Nichols	3.96
627	75	Lakeland	3.90
440*	75.5	Mulberry	3.88
441*	78	"	3.93
615*	..	Brewster	3.96
616*	..	"	4.08
617*	..	"	4.01
HARD-ROCK PHOSPHATE			
434	..	Dunnellon	3.76
435†	..	"	3.91
588*	77-80	Floral City	3.86
589*	77-80	"	3.79
590*	72.5	Benotis	3.48
591*	74	Inverness	3.77
623*	..	Hernando	3.93
624*	80	Dunnellon	3.85
625*	80	"	3.95
SOFT PHOSPHATE			
443	..	Gilchrist Co.	3.79
580	..	Ocala	1.23
581	..	"	1.04
LAND-PEBBLE PHOSPHATE MATRIX			
442‡	..	Mulberry	3.19

* Sample representing large shipment.

† Composite sample from various deposits.

‡ Material as mined. Contains phosphate, silica, clay, etc.

Twenty samples of land-pebble and nine of hard-rock phosphate were analyzed for fluorine. Fifteen of these samples represented shipments of 600–5,374 tons, and the others were good average samples of the various types and grades.

With the exception of two samples, Nos. 616 and 617, both from the same general locality, the figures in Table 1 show that the fluorine content of land-pebble phosphate varied from 3.86 to 3.98 per cent, the average for the twenty samples being 3.94 per cent. Furthermore, there does not appear to be any direct relation between the fluorine content and the tricalcium phosphate content of land-pebble phosphate.

Eldridge (10) reports 1.86 and 2.72 per cent fluorine in two samples of land-pebble phosphate containing 69 and 76 per cent calcium phosphate, respectively. Carnot (2) determined fluorine by passing silicon tetrafluoride into a solution of potassium fluoride and weighing the precipitated potassium silicofluoride. Using this method, he (7) found 3.36 per cent fluorine in a sample of land-pebble phosphate containing 77 per cent calcium phosphate. These figures show wide variations and are much lower than those obtained by the writers on the same grades of phosphate.

The results given in Table 1 show that the fluorine content of Florida hard-rock is, in general, somewhat lower than that of the same grade of land-pebble phosphate. The figures, which vary from 3.48 per cent fluorine in hard-rock phosphate containing 72.5 per cent calcium phosphate to 3.95 per cent in phosphate containing 80 per cent calcium phosphate, indicate that the fluorine content of hard-rock phosphate varies to a certain extent directly with the tricalcium phosphate content.

Eldridge (10) reports 2.29, 2.40, 2.66, 1.94, and 2.46 per cent fluorine in samples of hard-rock phosphate containing 74, 74, 77, 82.5, and 85 per cent calcium phosphate, respectively. Carnot (7), using the method previously mentioned, found 3.21 per cent fluorine in a sample of hard-rock phosphate containing 83.5 per cent calcium phosphate. Parrish and Ogilvie (16) give 2.14 per cent as the fluorine content of a sample of hard-rock phosphate containing 83 per cent calcium phosphate. Braun (1) reports 5.54–5.68 per cent fluorine in hard-rock phosphate, as determined by the Fresenius method¹. These figures show even wider variations than those reported for land-pebble phosphate and, except for those given by Braun, are considerably lower than the results obtained by the writers on approximately the same grades of phosphate. Braun's figures are, undoubtedly, much too high.

The composition of the so-called "soft phosphates" varies considerably in different samples. The fluorine content of three samples analyzed in the present investigation varied from 1.04 to 3.79 per cent. Eldridge (10)

¹ Quantitative Analysis, Vol. 1, p. 431. 1875.

and Carnot (7) report 2.58 and 3.19 per cent fluorine in samples containing 35.19 and 33.52 per cent phosphoric acid (P_2O_5), respectively.

The one sample of land-pebble phosphate matrix analyzed in this investigation contained 3.19 per cent fluorine. Eldridge reports 0.88 per cent fluorine in a sample containing 13.58 per cent phosphoric acid (P_2O_5).

FLUORINE CONTENT OF TENNESSEE PHOSPHATE ROCK.

At the present time the Tennessee phosphate deposits rank next in production to those of Florida in the United States. The two most important classes are the blue-rock and brown-rock phosphates, and of these two classes the brown-rock is by far the most important commercially at the present time.

Fourteen samples of brown-rock and five of blue-rock phosphate were analyzed for fluorine. Eight of these samples represented shipments of 400–2,400 tons, and the majority of the others were good average samples representative of the particular deposits and grades. The samples of brown-rock phosphate, except Nos. 564 and 587, which are from Wales, Tenn., are from the various mines operating in the Mt. Pleasant district. The samples of blue-rock phosphate are from two mines operating at Gordonsburg.

TABLE 2.

Fluorine content of Tennessee phosphate rock.

SAMPLE NO.	APPROXIMATE $Ca_3(PO_4)_2$ CONTENT	FLUORINE	COMMENTS
BROWN-ROCK PHOSPHATE			
	<i>per cent</i>	<i>per cent</i>	
573*	65	3.22	Considerable flint present
587	65–66	3.24	"Cone sand" from brown-rock phosphate washer
575*	66.5	3.32	Considerable flint present
577*	68	3.48	" " " "
...	69	3.56	Bureau of Standards, standard sample No. 56
564	72	3.62	
578*	72.5	3.60	
574*	73	3.49	
566	72–74	3.72	"Fertilizer grade" material
565	74–76	3.94	"Furnace grade" material
583	83	4.08	Carefully selected lump rock
584	..	3.78	" " " "
585	..	2.62	Run-of-mine material
586	..	3.89	High-grade washed and ground rock
BLUE-ROCK PHOSPHATE			
571*	62	3.29	
572*	65	3.37	
576*	68.5	3.71	
448	..	3.67	Sample from individual lump of rock
449	..	3.95	" " " " " "

* Sample representing large shipment.

The results given in Table 2 vary from 3.22 per cent fluorine in brown-rock phosphate containing 65 per cent calcium phosphate to 4.08 per cent in phosphate containing 80 per cent calcium phosphate. The average for thirteen samples, exclusive of No. 585, which is run-of-mine material, is 3.61 per cent fluorine. At the present time the commercial grades of Tennessee brown-rock phosphate, ordinarily used in the manufacture of fertilizers, usually contain about 68–75 per cent calcium phosphate. The average fluorine content of nine samples containing approximately these percentages of calcium phosphate is 3.67 per cent. As in the case of Florida hard-rock, the figures indicate that the fluorine content of brown-rock phosphate varies, to a certain extent, directly with the tricalcium phosphate content.

The figures on blue-rock phosphate indicate that this material contains more fluorine than is present in the same grades of brown-rock phosphate. The average for four samples, excluding No. 571, which is a somewhat lower grade than is ordinarily used in the manufacture of fertilizer at the present time, is 3.67 per cent.

The literature does not seem to contain any data on the fluorine content of the commercial grades of Tennessee phosphate rock.

THE FLUORINE CONTENT OF WESTERN PHOSPHATE ROCK.

Deposits estimated to contain approximately six billion tons of phosphate rock exist in the states of Idaho, Montana, Utah, and Wyoming. Owing to their distance from the principal fertilizer consuming districts, these deposits are worked only to a limited extent at the present time. However, they constitute one of the largest known reserves of phosphate rock in the world and in time undoubtedly will be of great value as sources of phosphate fertilizer for the middle-western states.

Eleven samples of Idaho and Wyoming phosphate rock were analyzed for fluorine. Three of these samples represented shipments of about 30 tons each, and the others were good average samples of the particular deposits and grades.

According to the results given in Table 3, the fluorine content of the eleven samples varied from 3.10 to 3.76 per cent, the average being 3.45 per cent. The figures indicate that the fluorine content of Western phosphate varies directly, to a certain extent, with the tricalcium phosphate content. Comparison of the figures in Tables 2 and 3 shows that in general the fluorine content of Idaho and Wyoming phosphate is somewhat higher than that of the same grades of Tennessee brown-rock phosphate.

Gale and Richards (12) report 0.40–0.66 per cent fluorine in four samples of Western phosphate containing 60–79 per cent calcium phosphate. Their low results are probably due to the use of the Berzelius gravimetric method in making the determinations. Wagner and Ross (22)

TABLE 3.
Fluorine content of western phosphate rock.

SAMPLE NO.	APPROXIMATE $\text{Ca}_3(\text{PO}_4)_2$ CONTENT	LOCATION OF DEPOSIT	FLUORINE
IDAHO PHOSPHATE ROCK			
492	<i>per cent</i> 63.5	Montpelier Canyon	<i>per cent</i> 3.34
493	66.5	"	3.39
489	67.5	Georgetown Canyon	3.49
494	72	"	3.70
490	72	Paris	3.76
550	"	3.43
454	Conda	3.40
WYOMING PHOSPHATE ROCK			
467*	.	Cokeville	3.10
468*	..	"	3.44
469*	...	"	3.51
491	63.5	"	3.39
BRITISH COLUMBIA PHOSPHATE ROCK			
582	...	Crow's Nest Pass Area	2.64

* Sample representing shipment of 30 tons.

have also pointed out the inaccuracy of the Berzelius method in the presence of large quantities of phosphate.

The sample of British Columbia phosphate, containing 2.64 per cent fluorine, is representative of a large deposit of rather low-grade material occurring in the Crow's Nest Pass district.

FLUORINE CONTENT OF NORTH AFRICAN PHOSPHATE ROCK.

At the present time the North African countries, Algeria, Egypt, Morocco, and Tunis, produce about 60 per cent of the world's phosphate rock and furnish about 80 per cent of the total quantity consumed in Europe. The deposits are widely distributed and vary markedly in their physical characteristics. With the exception of the Morocco deposits the phosphate is relatively low grade, usually containing less than 68 per cent calcium phosphate.

Thirteen samples¹ of phosphate from various deposits in North Africa were analyzed. The samples were probably fairly representative of the particular deposits and grades of phosphate.

¹ These samples, except No. 453, were furnished by the Établissements Kuhlmann, Paris, France.

TABLE 4.

Fluorine content of North African phosphate rock.

SAMPLE NO.	APPROXIMATE $\text{Ca}_3(\text{PO}_4)_2$ CONTENT	LOCATION OF DEPOSIT	FLUORINE
	<i>per cent</i>		<i>per cent</i>
551	58-63	Tebessa, Algeria	3.43
557	58-63	Tocqueville, Algeria	3.71
558	58-63	Rebiba, Algeria	3.65
559	58-63	Bordj-Redir, Algeria	4.16
560	58-63	Dyr, Algeria	3.35
562	58-63	M'Zaita, Algeria	3.68
552	58-63	Gafsa, Tunis	3.46
553	63-68	" "	3.77
556	58-63	Kalaa-Djerda, Tunis	3.48
561	65-70	M'Dilla, Tunis	3.72
555	58-63	Kosseir, Egypt	3.65
563	76-78	Morocco	4.28
453	72+	Morocco, Run-of-mine material	4.15

The results given in Table 4 show that the fluorine content of North African phosphate, containing 58-63 per cent calcium phosphate, varies from 3.35 to 4.16 per cent, the latter figure being higher than was found in any samples of phosphate rock from deposits in the United States. The highest fluorine content, 4.28 per cent, found in any phosphate rock was in the sample of high-grade Morocco phosphate, No. 563.

Dussert (9) reports analyses of samples from a number of the principal deposits in Algeria. The figures for fluorine vary from 0.99 to 3.16 per cent in phosphate containing 53-73 per cent calcium phosphate. Carnot (7) reports 2.01-3.78 per cent fluorine in commercial grades of phosphate rock from various deposits in Tunis and Algeria. Schucht (17) gives 3.30 per cent as the fluorine content of Gafsa phosphate. Parrish and Ogilvie (16) give 2.55 per cent as the fluorine content of a sample of high-grade Morocco phosphate from deposits about 60 miles from Casablanca. This figure is very much lower than the figures, 4.15 and 4.28 per cent fluorine, obtained by the writers on two samples of Morocco phosphate from the same general locality.

FLUORINE CONTENT OF SOME PHOSPHATE MINERALS.

Several samples¹ of apatite and other phosphate minerals were analyzed for fluorine, and the results are given in Table 5.

The fluorine content of five samples of fluoroapatite from deposits in Ontario, New York, and Mexico varied from 3.44 to 4.24 per cent. Carnot (6) reported that the highest percentage of fluorine that he found was 3.63 in a specimen from Knappenwand, Tyrol. Fritsch (11),

¹ These samples were furnished by the Mineral Division, U. S. National Museum, except the sample of triplite, which was furnished by E. P. Henderson, U. S. Geological Survey.

TABLE 5.

Fluorine content of some phosphate minerals.

SAMPLE NO.	MINERAL	LOCATION OF DEPOSIT	FLUORINE	COMMENTS
			<i>per cent</i>	
633	Fluorapatite	Renfrew County, Ontario	3.88	Large crystal
646	"	" " "	3.84	" "
647	"	" " "	3.89	" "
648	"	Hammond, St. Lawrence Co., N. Y.	4.24	" "
649	"	Durango, Mexico	3.44	Crystalline sample from iron ore deposit
634	Chloroapatite	Krageroe, Norway	0.17	
631	Wavellite	Mount Holly Springs, South Mountain, Pa.	3.95	
632	Amblygonite	Pala, San Diego County, Calif.	4.02	
...	Triplite	Sierra de Zapata, Catamarca Province, Argentine	7.72	Carefully selected crystals
629	Vivianite	Mullica Hill, N. J.	none	
630	Dufrenite	Rockbridge County, Va.	none	

quoting various authorities, gives 3.52, 3.31, and 3.13 per cent as the fluorine content of Ontario, Ottawa, and New York apatite, respectively.

Carnot (5) also reports analyses of four samples of wavellite, mineral aluminum phosphate, from Ireland and the United States, the fluorine content varying from 1.81 to 2.79 per cent. These figures are much lower than the results, 3.95 per cent fluorine, obtained by the writers on a sample of wavellite from Mount Holly Springs, Pa. This particular deposit is of interest because the mineral was used at one time as a raw material for the manufacture of elementary phosphorus (19).

FLUORINE CONTENT OF MISCELLANEOUS SAMPLES OF PHOSPHATE ROCK AND PHOSPHATIC MATERIALS.

Nauru and Ocean Islands phosphate rock is probably the highest grade material produced at the present time. The commercial product contains 85-90 per cent calcium phosphate and is exported principally to Australia and New Zealand.

Schucht (17), gives 2.00 and 2.20 per cent as the fluorine content of Nauru and Ocean Islands phosphate, respectively. Steel (18) reports varying small amounts of fluorine in 13 samples. Analyses given in Table 6 show 2.62 and 2.97 per cent fluorine, respectively, in two samples¹ of Nauru and Ocean Islands rock.

The South Carolina phosphate deposits were the first to be developed in this country, and for twenty years prior to 1888, when the first shipments of phosphate were made from Florida, they furnished practically all the mineral phosphate used for fertilizer purposes in the United

¹ These samples and the sample of Christmas Island phosphate were furnished by Cuming, Sm Co., Melbourne, Australia.

TABLE 6.

Fluorine content of miscellaneous samples of phosphate rock and phosphatic materials.

SAMPLE NO.	NATURE OF MATERIAL	FLUORINE	COMMENTS
		<i>per cent</i>	
450	Nauru Island phosphate rock	2.62	Representative sample of commercial material containing about 85-87 per cent $\text{Ca}_3(\text{PO}_4)_2$.
451	Ocean Island phosphate rock	2.97	Representative sample of commercial material containing about 88-90 per cent $\text{Ca}_3(\text{PO}_4)_2$.
452	Christmas Island phosphate rock	1.32	Representative sample of commercial material.
495	South Carolina phosphate rock	2.20	Museum sample of land rock.
650	"	3.43	"
643	Fish teeth from Florida land-pebble phosphate deposit	3.48	"
644	Animal teeth from Florida land-pebble phosphate deposit	1.76	"
645	Animal bones from Florida land-pebble phosphate deposit	3.29	"
653	Superphosphate prepared from Florida land-pebble phosphate containing 66-68 per cent $\text{Ca}_3(\text{PO}_4)_2$	1.86	Representative sample of commercial material containing 17-18 per cent P_2O_5 .
651	Double superphosphate prepared from Tennessee brown-rock phosphate	3.74	Representative sample of commercial material containing about 45-48 per cent P_2O_5 .
652	Double superphosphate prepared from Idaho phosphate rock	1.40	Representative sample of commercial material containing about 45-48 per cent P_2O_5 .

States. Owing to the high cost of mining, the low grade of the product, and competition from Florida and Tennessee, the industry declined steadily, and at the present time mining has ceased altogether despite the fact that the deposits are estimated to contain about nine million tons of phosphate.

Carnot (7), Fritsch (11), Schucht (17), and Vibrans (21), report figures varying from 1.00 to 3.50 per cent for the fluorine content of South Carolina phosphate. The results given in Table 6 show 2.20 and 3.43 per cent in two museum samples. The writers were unable to obtain samples representing commercial shipments.

The fluorine content of samples of fish teeth, animal teeth, and animal bones from Florida land-pebble phosphate deposits was 3.48, 1.76, and 3.29 per cent, respectively. Carnot (4) analyzed a number of fossil bones of various geological ages and reports 1.16-2.87 per cent fluorine in the ash. On the other hand, he found an average of only 0.20 per cent fluorine in the ash of human, animal, fish, and reptile bones of the modern period. He attributes the high fluorine content of the fossil bones to the infiltration of fluorine-bearing waters and to the combination of the

fluorine with the phosphate of lime, which seems more reasonable than to suppose that the bones of prehistoric animals originally contained such abnormally high percentages of fluorine.

RELATION BETWEEN THE PHOSPHORIC ACID (P_2O_5) AND THE FLUORINE CONTENT OF PHOSPHATE ROCK.

The writers have already pointed out that the fluorine content of Florida land-pebble phosphate is very uniform regardless of the commercial grade of the rock or the location of the deposit. On the other hand, the fluorine content of Tennessee and of Western phosphate appears to vary directly, to a certain extent, with the tricalcium phosphate content. The phosphoric acid (P_2O_5) fluorine ratio was determined on 15 samples of land-pebble, brown-rock, and Western phosphate, and the results are given in Table 7.

TABLE 7.

Relation between the phosphoric acid (P_2O_5) and the fluorine content of phosphate rock.

SAMPLE NO.	P_2O_5	FLUORINE	P_2O_5 -FLUORINE RATIO
FLORIDA LAND-PEBBLE PHOSPHATE			
	<i>per cent</i>	<i>per cent</i>	
436	32.14	3.91	8.22
437	33.28	3.94	8.45
438	33.58	3.90	8.61
439	34.42	3.87	8.89
440	34.90	3.88	9.00
441	35.97	3.93	9.15
			Average 8.72
TENNESSEE BROWN-ROCK PHOSPHATE			
573	30.09	3.22	9.35
575	30.62	3.32	9.22
574	33.38	3.49	9.56
			Average 9.38
WESTERN PHOSPHATE			
492	29.19	3.34	8.74
491	29.35	3.39	8.66
493	30.84	3.39	9.10
489	31.08	3.49	8.91
490	33.23	3.76	8.84
494	33.25	3.70	8.99
			Average 8.87

The results show that the phosphoric acid (P_2O_5)-fluorine ratio increases in Florida land-pebble phosphate as the phosphoric acid content increases. This is to be expected in view of the uniformity of the fluorine content of this material. Although the number of determinations made were insufficient to justify the drawing of definite conclusions regarding

the phosphoric acid (P_2O_5)-fluorine ratio in Tennessee brown-rock and Western phosphate, the results obtained thus far indicate that the ratio is higher in the former than in the latter. Furthermore, the ratios may be considered as fairly constant for each of these two types of phosphate. A further study of the relation between the phosphoric acid and the fluorine content of phosphate rock is planned.

If the formula, $3 Ca_3(PO_4)_2 \cdot CaF_2$, usually ascribed to fluoroapatite, is correct, then the phosphoric acid (P_2O_5)-fluorine ratio in this material should be 11.217. In this event, it is evident that nearly all the samples of phosphate rock analyzed in the present investigation contain considerably more fluorine than would be the case if all the phosphoric acid present was in the form of fluoroapatite.

VOLATILIZATION OF FLUORINE IN THE MANUFACTURE OF SUPERPHOSPHATE.

According to Schucht (17), one writer reports that two-thirds of the fluorine present in phosphate rock is volatilized in the manufacture of superphosphate, while one-third remains in the superphosphate as calcium fluoride. It is stated by another investigator that about 50 per cent of the fluorine is volatilized, about 30 per cent remains undecomposed, and about 20 per cent is mechanically absorbed as hydrofluosilicic acid in the superphosphate. Braun (1) also concludes that about 50 per cent of the fluorine is volatilized from phosphate rock in the manufacture of superphosphate. Parrish and Ogilvie (16) state that in actual practice 13.6–19.2 pounds of sodium fluosilicate is recovered per ton of phosphate rock dissolved.

The writers have analyzed several samples of superphosphate representative of a large quantity of commercial material manufactured from Florida land-pebble phosphate containing 66–68 per cent calcium phosphate. The average fluorine content of the different samples was 1.86 per cent calculated on the moisture-free basis (see Table 6, Sample 653). The average fluorine content of the samples as received was 1.80 per cent, the samples containing an average of about 4 per cent moisture. The particular plant manufacturing this material obtains about 1 ton of superphosphate from 1200 pounds of phosphate rock. If 3.94 per cent is taken as the average fluorine content of Florida land-pebble phosphate, then 1200 pounds of the rock contain 47.28 pounds of fluorine. One ton of the superphosphate contains 36 pounds of fluorine. From these calculations it is evident that approximately 24 per cent of the fluorine originally present in the phosphate rock is volatilized in the manufacture of superphosphate. It is probable that the percentage of fluorine volatilized will vary somewhat with the different grades and types of phosphate rock and also with the different methods used in the manufacture of superphosphate.

Approximately two million tons of Florida land-pebble phosphate, containing about 78,800 tons of fluorine, is used annually in the United States for the manufacture of superphosphate. Approximately 400,000 tons of Tennessee phosphate rock, containing about 14,680 tons of fluorine, is also used for this purpose. If it is assumed that in the manufacture of superphosphate 25 per cent of the fluorine is volatilized and can be recovered, then the phosphate rock used annually in the United States for this purpose represents a potential supply of approximately 25,000 tons of pure fluorine, equivalent to 41,270 tons of sodium fluosilicate, which, according to current market quotations, would have a value of \$3,714,300.

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The writers wish to express their appreciation of the kindness of a number of the phosphate mining companies and fertilizer manufacturers and also of several interested individuals who furnished the majority of the samples of phosphate rock.

SUMMARY.

(1) A study was made of the fluorine content of the various commercial grades and types of phosphate rock from deposits in the United States and several foreign countries.

(2) The fluorine content of 20 samples of different commercial grades of Florida land-pebble phosphate varied from 3.86 to 4.08 per cent, the average being 3.94 per cent.

(3) The fluorine content of 13 samples of Tennessee brown-rock phosphate varied from 3.22 to 4.08 per cent. The average for 9 samples of the usual commercial grades, containing 68–75 per cent calcium phosphate, was 3.67 per cent fluorine. Four samples of the usual commercial grades of Tennessee blue-rock phosphate contained 3.37–3.95 per cent fluorine, the average being 3.67 per cent.

(4) Ten samples of phosphate rock from Idaho and Wyoming, containing 63.5–72 per cent calcium phosphate, contained 3.34–3.70 per cent fluorine.

(5) Thirteen samples of phosphate rock from various deposits in North Africa contained 3.35–4.28 per cent fluorine.

(6) Figures are given on the fluorine content of apatite and several other phosphate minerals, and also of Nauru, Ocean and Christmas Islands, and South Carolina phosphate rock.

(7) The phosphoric acid (P_2O_5)-fluorine ratio of Florida land-pebble phosphate increases with increase in the phosphoric acid content of the rock, but preliminary data indicate that the ratio is fairly constant in different grades of Tennessee brown-rock and Western phosphate.

(8) Analyses of superphosphate, manufactured from Florida land-

pebble phosphate, indicate that about 25 per cent of the fluorine present in phosphate rock is volatilized in the manufacturing process.

(9) The fluorine volatilized annually in the manufacture of superphosphate in the United States represents a potential source of approximately 25,000 tons of pure fluorine, the greater part of which can be recovered in the form of fluosilicates.

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ESTIMATION OF BUTTERMILK OR MILK PRODUCT IN A MIXED FEED BY DETERMINATION OF THE LACTOSE PRESENT.

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A growing demand for dried buttermilk for use in poultry and certain other stock feeds has been stimulated by numerous favorable reports on this material from the State Experiment Stations. Manufacturers of mixed feed were not slow to include this new ingredient in their products and to emphasize unduly its presence in their advertising.

This situation makes it desirable to have a method that will show the presence or absence of dried buttermilk and indicate as nearly as possible the quantity present. The one reported in this paper is based on the estimation of lactose contained in the finished feed.

PART I. LACTOSE IN DRIED BUTTERMILK.

The quantity of lactose in buttermilk is variable because it is affected by the season of the year and by the treatment it receives during manufacture. Lactic acid bacteria change lactose to lactic acid, thereby decreasing the amount of lactose and increasing the acidity. This activity is greater in summer than in winter. It is the belief of some butter makers that cream containing 0.2 per cent acidity produces a commodity with the best flavor. In winter cream usually can be kept to this limit of acidity; in summer, however, it is often necessary to use a "neutralizer".

The lactose content of dried buttermilk may run as low as 7.1 per cent and as high as 47 per cent. This variation is so wide that when the percentage of lactose is used to estimate the amount of buttermilk in a mixture, the figure that will give the maximum, average, or minimum lactose content, as the case may require, must be used. For example, a fair figure for estimating the maximum buttermilk content would be 8 per cent, for average content 26 per cent, and for a minimum buttermilk content 40 per cent. Then if the analyst uses 8 per cent as the factor the report might read "not over . . . per cent dried buttermilk"; if he uses 26 per cent as the factor it might read, "about . . . per cent dried buttermilk"; and if he uses 40 per cent as the factor it would read "at least . . . per cent dried buttermilk".

Thus it is evident that a feed may contain more or less buttermilk than represented; nevertheless, by using the factors, the determination serves as a fairly accurate estimate of the amount present. For instance, the results obtained when the 8 per cent factor is used in a control method in the enforcement of a feed law may indicate more buttermilk than actually was added. This is the case when the lactose content of the

particular buttermilk used is high. The calculated amount of buttermilk in the feed approaches the correct value as the lactose content in the buttermilk added approaches the factor used.

Numerous methods are available for determining lactose in milk products, but since no record of the use of the lactose figure in estimating the amount of a milk product in materials such as mixed feeds was found the writer formulated the following modification of the picric acid method and used it in analyzing a large number of buttermilk samples.

PROCEDURE.

For dried buttermilk, dried skim milk, or whole milk, grind the sample to pass a 40-mesh sieve. After washing with 20 cc. of sulfuric ether, take 2 grams of sample, place in a 300 cc. volumetric flask, and add about 200 cc. of distilled water, taking care that no lumps form. Heat the mixture in a boiling water bath for 30 minutes, shaking occasionally to bring the lactose into solution.

Cool and make up to mark. Stir the sample thoroughly in a suitable beaker until no lactose remains undissolved and enclosed in globules formed of casein, albumin, or fat. Filter the milk solution through a double thickness of filter paper placed in a Büchner funnel. The filtrate should be either clear or opalescent.

Saturate a small portion of the filtrate with dry picric acid crystals to precipitate the proteins in solution and also to provide the picric acid filtrate which contains the lactose. Add 1-3 cc. of the picric acid filtrate to a Nessler tube, taking care that no solution touches the sides in transferring. Add 1 cc. of 22 per cent sodium carbonate and immerse the tube in a boiling water bath for 15 minutes. Cool the solution and dilute to nearly the same color as a standard that contains 0.0002 gram of lactose per cubic centimeter. Take 3 cc. of this standard solution plus 1 cc. of the carbonate solution, heat with the sample, and dilute to 30 cc. Run a new standard with each lot of unknowns, because the color developed by the addition of alkali changes after standing. Make a comparison in any good colorimeter.

When the gravimetric method for determining sugars is preferred, transfer a 150 cc. aliquot of the clear filtrate from the Büchner funnel filtration into a 200 cc. volumetric flask; add 10 cc. of 15 per cent sodium tungstate solution, 5 drops of thymol blue, and then concentrated sulfuric acid drop by drop until the solution turns pink. Then add ten drops more of sulfuric acid in order to be sure of the proper pH (1.5-2.0). Make up to mark and allow to stand overnight. Filter. A 25 cc. aliquot is generally the proper quantity to use for the Munson and Walker method¹.

Results by the picric acid method are calculated by the following formula:

$$\begin{aligned}
 A &= \text{Original volume (300 cc.)}, \\
 B &= \text{Quantity taken for reading (3 cc.)}, \\
 C &= \text{Dilutions of } B \quad (-), \\
 D &= \text{Dilutions of standard (30 cc.)}, \\
 R_1 &= \text{Reading of standard}, \\
 R_2 &= \text{Reading of sample}, \\
 0.0006 &= \text{Weight of lactose in grams in 3 cc. of standard}, \\
 W &= \text{Weight of sample taken}, \\
 0.9500 &= \text{Factor for anhydrous lactose}, \\
 X &= \frac{A \times C \times R_1 \times 0.0006 \times 100}{B \times D \times R_2 \times W} = \text{Percentage of lactose in sample.} \\
 X \times 0.95 &= \text{anhydrous lactose in sample.}
 \end{aligned}$$

¹ *Methods of Analysis*, A. O. A. C., 1925, 190.

TABLE 1.

Results of analyses of buttermilk manufactured in the month of June by the roller process.

SAMPLE NO.	MOISTURE	FAT	PROTEIN	LACTOSE	ASH	LACTIC ACID
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
87	7.41	2.85	31.44	8.17	14.20	7.07
88	7.81	2.75	33.13	7.10	14.26	6.93
89	7.57	5.68	32.25	15.45	12.29	4.41
90	9.37	5.60	31.38	23.73	12.40	4.46
91	9.49	7.09	34.25	13.47	10.96	4.60
92	7.58	5.53	30.38	13.19	10.13	3.72
97	7.88	4.80	33.31	9.00	14.23	8.55
98	8.64	9.29	34.06	9.00	12.21	8.93
99	11.69	5.75	30.38	20.28	12.61	5.48

High ash figures in Table 1 illustrate the effect of using neutralizers in preparing the cream for churning. The use of neutralizers, together with the conversion of lactose to lactic acid by fermentation, also accounts for the low lactose figure. Attention is also called to the high moisture content, which explains why dried buttermilk becomes lumpy when stored.

TABLE 2.

Results of analyses of buttermilk manufactured during the months of February and March by the roller process.

SAMPLE NO.	MOISTURE	FAT	PROTEIN	LACTOSE	ASH	LACTIC ACID
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
104	4.89	4.72	32.50	25.34	10.75	6.00
107	4.54	9.08	34.00	22.55	11.76	3.16
108	6.86	5.32	33.19	31.66	8.22	2.69
109	4.61	6.60	32.75	32.01	9.08	4.14
129	4.90	0.59	34.94	42.17	7.79	1.30
130	5.72	6.00	33.13	27.25	10.78	4.28
131	4.98	2.16	32.50	41.13	8.92	0.77
132	4.75	1.59	35.50	26.48	10.72	4.69
134	2.52	4.06	34.31	26.39	10.68	4.37
140	3.73	5.98	32.94	33.05	10.37	3.21
141	5.24	4.94	31.00	34.24	8.72	3.58
142	4.61	5.48	31.63	33.34	9.96	3.39
143	4.37	4.28	30.63	35.20	9.37	3.86
144	4.29	7.36	32.81	29.46	9.12	4.04
145	6.19	5.49	34.31	28.32	10.11	4.32
146	4.48	6.30	32.75	33.34	9.67	4.18
165	4.89	0.68	33.75	30.89	9.67	4.15
370	4.58	5.14	32.19	35.19	8.94

Noting the same points in Table 2 as in Table 1, it will be found that the moisture and ash results are low and the lactose figure high. This is because the low activity of lactic-acid-producing organisms has obviated the use of neutralizers.

Table 3 illustrates the agreement of results obtained by the picric acid method with those obtained by the copper reduction method.

Table 4 illustrates how closely it is possible to approach the theoretical figure when the proposed method is used if the lactose content of the buttermilk used is known.

TABLE 3.

Results of analyses of buttermilk comparing the picric acid method with the copper reduction method¹.

SAMPLE NO.	PICRIC ACID METHOD	COPPER REDUCTION METHOD
	<i>per cent</i>	<i>per cent</i>
134	26.39	25.77
140	32.48	33.05
142	33.34	33.52
144	29.46	30.08
146	33.34	33.60
165	30.89	31.52
222	20.44	20.00

TABLE 4.

Comparison of results calculated as buttermilk with the actual amount of buttermilk added to a mixed feed.

BUTTERMILK	
ADDED	FOUND
<i>per cent</i>	<i>per cent</i>
10	9.78
10	10.34
10	9.89
10	9.46
8	8.26
5	4.33
5	4.11
5	4.70
1	1.30
1	0.90

PART II. ESTIMATION OF DRIED BUTTERMILK FROM LACTOSE CONTENT.

The preliminary work on a method for estimating lactose in a mixture of feeds consisted in preparing a feed similar to those on the market and then analyzing samples to which varying quantities of buttermilk having a known lactose content had been added. The quantity of buttermilk in the feed was calculated by means of the lactose figure thus found and compared with the theoretical.

The proposed method is based on the fact that lactose is not fermented by yeast, as are the plant sugars commonly found in mixed feeds, and therefore may be determined after the other sugars have been eliminated. Apparently the only feedstuffs that contain interfering substances that make the method inapplicable are cottonseed meal and soybean meal. However, most buttermilk feeds do not have these ingredients, and therefore the method should have wide application.

The difficulty experienced at first in obtaining a satisfactory blank was found to be due to the incomplete elimination of the proteins. When greater care was taken in keeping the proper temperature during fermentation, results were much closer to the theoretical. It was found that during warm days the plant sugars were completely fermented over-

¹ *Methods of Analysis*, A. O. A. C., 1925, 189.

night, but during the cooler weather the time required was longer and the necessity of keeping the proper temperature by artificial means became apparent.

Details of the method are as follows:

PROPOSED METHOD.

REAGENTS.

- (a) *Picric acid*.—Dry and of good grade.
- (b) *Sodium carbonate*.—22 per cent solution.
- (c) *Picric acid*.—Saturated and containing 0.0002 gram of lactose as the standard. (This solution will keep indefinitely in the dark.)
- (d) *Alumina cream*.
- (e) *Sodium tungstate*.—15 per cent solution.

PROCEDURE.

Grind the feed until it passes a 40-mesh sieve and weigh out a 10 gram sample. Extract with 20 cc. of ether, place in a 300 cc. volumetric flask, and add 200 cc. of distilled water. Heat the flask and contents in the steam bath for exactly 30 minutes, shaking frequently to dissolve all the lactose. After cooling make up to mark.

Filter through a Büchner funnel into a flask and then transfer 150 cc. of the filtrate to a 200 cc. volumetric flask. Add 20 cc. or more of alumina cream, make up to mark, and filter. Pipet 150 cc. of the clear filtrate into a 300 cc. Erlenmeyer flask, boil, cool, and add 0.75 gram of compressed or brewer's yeast. Allow the mixture to ferment overnight at 25°–30°C. (In cool weather at least 2 days may be necessary.)

Boil off the carbon dioxide and alcohol and transfer to a 100 cc. volumetric flask. Add 5 cc. of sodium tungstate and 5 drops of thymol blue. Acidify, drop by drop, with concentrated sulfuric acid until a pink color appears, and then add 10 drops more. (The pH of the solution should be 1.5–2.0 for a complete precipitation.) Make up to mark and let stand overnight. If no precipitate appears, add 10 drops more of acid and allow to stand overnight. Filter the next morning. (The filtrate should be clear.) Saturate a portion of the filtrate (30 cc.) with picric acid, allow to stand 20 minutes, shaking frequently, and refilter.

Place 10 cc. of the filtrate in a Nessler tube so carefully that not even a drop touches the sides in the operation, add 3 cc. of the 22 per cent carbonate solution, and heat in the steam bath for 15 minutes with a standard tube containing 3 cc. of the picric acid solution plus 1 cc. of sodium carbonate. (The color that develops depends on the quantity of sugar present.)

After cooling, dilute the solution to a shade a trifle darker than the standard, the latter being diluted to 30 cc. Run a standard with each set of unknowns because the color changes after standing.

The Bürker colorimeter is satisfactory for making comparisons.

The lactose content and the amount of buttermilk it represents is figured by the following formula:

W = weight of sample.

$$X = \frac{300}{150} \times \frac{200}{150} \times \frac{150}{100} \times \frac{100}{10} \times \frac{\text{dilution of sample}}{\text{dilution of standard}} \times \frac{\text{reading of standard}}{\text{reading of sample}} \times \frac{0.0006 \times 100}{W(10)}$$

X = per cent of lactose.

$$Y = \text{per cent of buttermilk in feed} = \frac{X \times 100}{B}$$

B is the factor (8, 26, or 40) for lactose in the buttermilk used.

Multiply *Y* by 0.975 to allow for the volume occupied by sample.

When using the gravimetric method at least 50 cc. of the clear filtrate would be required.

TABLE 5.

Results of analysis of two commercial buttermilk feeds said to contain 5 per cent and 4 per cent, respectively.

SAMPLE NO.	LACTOSE per cent	USING 40% LACTOSE FACTOR- BUTTERMILK PRESENT	USING 8% LACTOSE FACTOR- BUTTERMILK PRESENT
		per cent	per cent
47814	0.31	0.76	3.76
50906	0.47	1.13	5.70

It would be concluded from the results given in Table 5 for Sample No. 47814 that the feed contained less buttermilk than was declared by the manufacturer, while in the case of Sample No. 50906, the amount of buttermilk shown to be present is somewhere between the minimum and maximum figures and substantiates his claim. When a claim for the amount of added buttermilk falls outside these two figures, it is reasonable to conclude that the amount declared on the label is not correct.

Possibly the principles used in the solution of this question would apply to the problems of the determination of milk in bread, of milk in milk chocolate, and of sucrose in cheese. In examining these products the sugars should be brought in solution, the proteins precipitated, the total sugars determined if this figure is desired, and then the solution should be fermented and the lactose determined. The sucrose would be determined by difference. In such products the results will have a greater degree of accuracy than in buttermilk in feeds, because there is no fermentative destruction of lactose in the sweet milk used.

SUMMARY AND CONCLUSIONS.

1. A modified picric acid method has been developed for estimating lactose.
2. A method for the estimation of buttermilk added to a mixed feed is offered for control purposes.
3. A milk product can be detected in mixed feeds by this method in quantities as low as one-half of one per cent, if a known buttermilk is used.
4. While the quantity of buttermilk in a mixed feed cannot be determined with the accuracy of a gravimetric method, its presence may be established within certain limits.
5. If a colorimeter is not available, the copper reduction method for sugar may be used, the only difference being that more sugar solution is necessary for the determination.

6. The usefulness of the picric acid method lies in its accuracy in determining small quantities of sugar.

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DETERMINATION OF CITRIC ACID IN FRUITS AND FRUIT PRODUCTS.

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In a previous paper¹ the authors showed that citric acid in pure solution can be accurately determined as pentabromacetone. The present paper deals with the application of the method to the determination of citric acid in fruits and fruit products.

The conversion of citric acid into pentabromacetone being primarily an oxidative procedure, it follows that the required quantities of the oxidizing agents, bromine and potassium permanganate, will depend

¹ *This Journal*, 1927, 10: 264.

upon the sugar content of the material under examination. The official method¹ for the determination of citric acid in fruit products directs the removal of interfering sugars before proceeding with the conversion into pentabromacetone, but the use of barium hydroxide specified is unsatisfactory owing to the time required for refluxing and the subsequent manipulations necessary to obtain the free acid.

Various other means of separating the acid from the sugars were tried. Alcohol is an excellent solvent for most fruit acids, but it also partially dissolves sugars. Advantage was taken of the fact that when ether is added to the alcoholic extraction the solubility of the sugars is decreased but the solubility of the acids is not materially affected. A large number of experiments with alcohol-ether extraction showed fairly good recovery of the acid. However, the removal of the alcohol by evaporation, in the presence of sulfuric acid, causes esterification and therefore a loss of citric acid because, as has been shown, the esters formed are not converted into pentabromacetone. It was found that acetone extraction gave better results than alcohol-ether extraction, but the separation of acid from sugars by any extraction method is of necessity time-consuming and laborious. It was decided, therefore, to investigate the direct determination of citric acid, omitting the removal of sugars.

As a basis for the study an apple jelly that had been prepared in the laboratory was selected; it was presumed to be free from citric acid and had a high sugar and pectin content. It soon became evident, however, that the procedure outlined in the previous paper was not suitable for a material containing large quantities of sugar. Large quantities of potassium permanganate were necessary for the oxidation, and then the reaction was not controllable. Results obtained by using phosphoric acid instead of sulfuric acid showed a better control of the oxidation. Since some sulfuric acid, however, is necessary for the complete removal of manganese dioxide no advantage was gained.

Observations on the formation of pentabromacetone led to the conclusion that under controlled conditions the compound forms quickly and that it is stable. According to Browne² bromine readily oxidizes dextrose into hexonic acid. It is evident, therefore, that in the presence of such large quantities of dextrose as are found in jams and jellies through inversion of sucrose, the bromine that is required for the conversion of citric acid is diverted to the formation of hexonic acid. With this fact in mind, experiments were conducted in which larger quantities of potassium bromide were used to stabilize the reaction. The increase of potassium bromide not only necessitated an increase in the quantity of sulfuric acid over that used in the procedure described in the previous

¹ *Methods of Analysis*, A. O. A. C., 1925, 215.

² *Handbook of Sugar Analysis*, 1st ed., p. 363. 1912.

paper, but it also called for a readjustment of the entire reaction, as it is obvious that a change in the quantities of reagents affects the solubility of pentabromacetone. It was found that increasing the quantities of sulfuric acid and potassium bromide decreased the pentabromacetone, but the reason was not apparent. Evaporation of an ether extraction of the filtrate obtained from the precipitated pentabromacetone yielded material quantities of an oily substance which may have been a decomposition product of pentabromacetone. Attempts to identify it, however, were unsuccessful. While the increased loss of pentabromacetone is regrettable, the use of larger quantities of potassium bromide and sulfuric acid makes it comparatively easy to obtain duplicable results, and this was impossible before.

As was pointed out in the previous paper, the official method is ambiguous in regard to the oxidation with potassium permanganate, and particularly so with respect to the completion of the reaction. It was believed that better results would be obtained if the directions specified a definite quantity of solids for the final determination and thereby made the volume of permanganate constant. Therefore, in order to determine the quantity of solids that could be used without disturbing the control of the procedure, numerous experiments with varying quantities of solids (apple jelly and invert sugar) were made. It was found that 12.5 grams of solids is the limit for maximum recovery of pentabromacetone. Accordingly, this quantity was chosen as a basis for subsequent work.

It seems unnecessary to give the data obtained in the experiments that were made to eliminate some of the difficulties mentioned; it is sufficient to state that after a thorough investigation the procedure described near the end of this paper was formulated.

For the purpose of determining the loss of citric acid by the direct bromination-oxidation procedure in a material high in sugar, experiments on apple jelly were made. Since 18.2 grams of the jelly contained 12.5 grams of solids, this quantity was employed for the determinations.

In all the tables the data refer to 100 cc. of solution obtained after saponification.

It will be noted in Table 1 that for the quantities of citric acid up to 80 mg. the percentage loss decreases with the increase of the citric acid present. For the larger quantities the losses are fairly constant.

In the procedure described in the previous paper a correction factor of 1.7 mg. of citric acid for every 100 cc. reaction mixture was introduced. In the procedure given in this paper the quantities of the various reagents necessary for the determination are fixed, and thus the correction factor is made a constant, as was stated previously.

TABLE 1.
Loss of citric acid in apple jelly containing varying quantities of added citric acid.

CITRIC ACID ADDED	CITRIC ACID DETERMINED	CITRIC ACID LOST	
mg.	mg.	mg.	per cent
25.9	18.5 17.8 17.2 Average 17.8	8.1	31.3
41.3	33.7 32.1 32.5 Average 32.8	8.5	20.6
51.6	41.8 41.6 42.4 Average 41.9	9.7	18.8
82.6	70.3 71.0 70.5 Average 70.6	12.0	14.5
102.8	90.5 90.0 89.3 Average 89.9	12.9	12.5
123.9	107.4 109.1 105.0 Average 107.2	16.7	13.5
153.8	134.7 134.0 Average 134.4	19.4	12.6
199.0	178.0 177.7 Average 177.9	21.1	10.6

In Table 2 the data necessary for the determination of this correction factor are presented. They were obtained on 100 cc. of a 12.5 per cent invert sugar solution containing varying quantities of added citric acid.

It will be noted in Table 2 that with the exception of the first determination (24.8 mg. of added citric acid) the percentage loss of citric acid is fairly constant. If the average of the five remaining results is used, it is necessary to add 0.122 gram for each gram of citric acid determined to compensate for the loss involved. This is equivalent to a correction factor of 1.14.

Since, as has been stated, this correction factor is a constant, the true citric acid content is obtained by multiplying the citric acid determined (referred to as "a" in the following tables) by 1.14.

When this correction factor is applied to the average results presented in Table 1, the following corrected data are obtained.

TABLE 2.

Loss of citric acid from invert sugar solutions containing varying quantities of added citric acid.

CITRIC ACID ADDED	CITRIC ACID DETERMINED	CITRIC ACID LOST	
		mg.	per cent
24.8	18.9	5.4	21.8*
	20.0		
	19.3		
	Average 19.4		
49.5	43.3	6.4	12.9
	43.3		
	42.7		
	Average 43.1		
73.9	63.1	11.0	14.8
	62.4		
	63.3		
	Average 62.9		
98.9	87.9	10.7	10.9
	88.2		
	88.6		
	Average 88.2		
156.2	138.1	18.1	11.6
	138.7		
	137.5		
	Average 138.1		
207.4	186.4	22.9	11.0
	182.7		
	184.4		
	Average 184.5		
			Average 12.2

* Not included in average.

TABLE 3.

Results in Table 1 corrected for loss of citric acid by applying the factor 1.14.

CITRIC ACID ADDED	CITRIC ACID DETERMINED a	CITRIC ACID CORRECTED 1.14a	ADDED CITRIC ACID	
			Present	Found
mg.	mg.	mg.	per cent	per cent
25.9	17.8	20.3	0.142	0.112
41.3	32.8	37.4	0.227	0.206
51.6	41.9	47.8	0.284	0.263
82.6	70.6	80.5	0.454	0.443
102.8	89.9	102.5	0.565	0.563
123.9	107.2	122.2	0.681	0.672
153.8	134.4	153.2	0.846	0.842
199.0	177.9	202.8	1.093	1.114

For the smaller quantities of citric acid the corrected figures fall short of the true citric acid content, while for the larger quantities they are acceptable. From the data given it is apparent that for satisfactory

results a quantity of acid equal to or exceeding 80 mg. is necessary. It would seem, therefore, that when the quantity of citric acid is less than 80 mg., more acid should be added to the portion taken for the final determination.

The effect on the determination of small quantities of the acid of an addition of citric acid is shown by the data given in Table 4. The samples on which the first five determinations were made were prepared by adding 12.4 mg. of citric acid to 35 grams of the material (25 grams of solids). In the case of the second determination on the blackberry jam, 40.8 mg. of citric acid was added to 35 grams of the material. In the preparation of the sample a dilution to 200 cc. was made, and 100 cc. of this solution was used for the determination; therefore, the figures given in columns 2 and 3 represent only one-half of the original material.

TABLE 4.

Effect of added citric acid on the determination of small quantities of citric acid.

MATERIAL	WEIGHT	CITRIC ACID PRESENT	CITRIC ACID ADDED IN FINAL DETERMINATION	CITRIC ACID DETERMINED ^a	CITRIC ACID CORRECTED 1.14a	CITRIC ACID	
						Present	Found
	<i>grams</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>per cent</i>	<i>per cent</i>
Apple jelly	17.5	6.2	60.0	55.8	63.6	0.035	0.014
				53.7	61.2		
				54.9	62.6		
				Average	62.5		
Apple jelly	17.5	6.2	100.0	92.4	105.3	0.035	0.026
				90.6	103.3		
				92.3	105.2		
				Average	104.6		
Apple jam	17.5	6.2	60.0	55.3	63.0	0.035	0.013
				54.0	61.6		
				54.4	62.0		
				Average	62.2		
Apple jam	17.5	6.2	100.0	93.2	106.2	0.035	0.037
				92.3	105.2		
				94.8	108.1		
				Average	106.5		
Blackberry jam	17.5	6.2	60.0	57.4	65.4	0.035	0.022
				55.1	62.8		
				55.6	63.4		
				Average	63.9		
Blackberry jam	17.5	20.4	40.7	51.4	58.6	0.116	0.104
				51.8	59.1		
				Average	58.9		

The results given in Table 4 show quite conclusively that the addition of citric acid is necessary in the determination of small quantities of the acid; accordingly, a provision for such addition is made in the proposed

procedure. This method, which is based on the data obtained in this investigation, makes possible the accurate determination of citric acid in products having a high sugar content. The essential requirements are a constant solids content in the portion taken for the determination (12.5 grams in 100 cc.) and a quantity of citric acid equal to approximately 100 mg. A larger quantity of citric acid does not affect the accuracy of the procedure, but it may make difficult the filtration and subsequent drying of the pentabromacetone.

Information regarding the approximate citric acid content of the material to be examined is usually available. Citrus fruits; jams and jellies prepared from strawberries, raspberries, loganberries, currants, blueberries, cranberries, and pomegranates; and tomato products normally contain sufficient citric acid so that no addition is necessary. If this information is lacking it will be necessary to make a preliminary examination to establish the approximate citric acid content of the product before finally deciding the quantity of acid to be added.

PROPOSED METHOD FOR THE DETERMINATION OF CITRIC ACID IN FRUIT AND FRUIT PRODUCTS.

PREPARATION OF SOLUTION.

Prepare a solution which will contain approximately 25 per cent of solids, either by diluting with water or adding sucrose. If alcohol is present, completely remove by evaporation. Place 100 grams of the adjusted solution on the steam bath for 15 minutes. Immediately neutralize with 20 per cent potassium hydroxide solution, adding 5 cc. in excess, and allow to stand overnight. Add 40 cc. of a 43 per cent sulfuric acid solution and heat to 50°C. on the water bath for 15 minutes. Cool, transfer to a 200 cc. volumetric flask, make to mark, and filter. The portion required for the determination, 100 cc. of the above filtrate, should contain approximately 100 mg. of citric acid. If the 100 cc. of solution does not contain the required quantity, add pure citric acid. If large quantities of citric acid are present, it will, of course, be necessary to use a smaller quantity of material for the adjustment.

REAGENTS.

Potassium bromide solution.—Dissolve 50 grams of potassium bromide in water and dilute to 100 cc.

Potassium permanganate solution.—Dissolve 5 grams of potassium permanganate in water and dilute to 100 cc.

Ferrous sulfate solution.—Dissolve 40 grams of ferrous sulfate in 100 cc. of water containing 1 cc. of concentrated sulfuric acid.

DETERMINATION.

Transfer 100 cc. of the prepared filtrate to a 500 cc. Erlenmeyer flask, add about 0.3 gram of purified asbestos and 25 cc. of the potassium bromide solution, and hold at 48°–50°C. for 30 minutes. Add 125 cc. of the potassium permanganate solution in three portions in rapid succession, shaking after each addition¹. Do not allow the tem-

¹ NORG.—When the reaction mixture is shaken violently in a stoppered flask, the pentabromacetone is precipitated in a form which facilitates filtration. However, the pressure developed is so great that unless it is released at frequent intervals the strain may cause the breaking of the flask.

perature to rise above 55°C. Hold at this temperature for 5 minutes after the first portion of the permanganate solution is added and immediately add 30 cc. of the ferrous sulfate solution to remove the manganese dioxide. Cool, and without agitating further place in a refrigerator overnight. Decant onto a thin, tightly tamped pad of asbestos in a Gooch crucible, transferring the precipitate to the crucible with a portion of the clear filtrate. Wash the contents of the crucible at once with three portions of 20 cc. each of ice-cold sulfuric acid (1 + 100) and three portions of 20 cc. each of ice-cold water. Dry the precipitate to constant weight over sulfuric acid in a vacuum desiccator, protecting from strong light. Weigh, and remove the pentabromacetone with three portions of 20 cc. each of 95 per cent alcohol and three portions of 20 cc. each of ether. Again dry and weigh. Multiply the weight of pentabromacetone by 0.424 to obtain anhydrous citric acid. Correct for loss of citric acid by multiplying the weight of the citric acid determined by the factor 1.14. If citric acid has been added, make allowance. (In order to expedite the process, the precipitate may be dried in a current of air passed through sulfuric acid.¹)

The proposed method was applied to a variety of fruits and fruit products. The results obtained are given in Table 5. While an explana-

TABLE 5.

Results obtained on fruit and fruit products when proposed method was used.

MATERIAL	WEIGHT	CITRIC ACID PRESENT	CITRIC ACID ADDED IN FINAL DETERMINATION	CITRIC ACID DETERMINED ^a	CITRIC ACID CORRECTED 1.14a	CITRIC ACID	
						Present	Found
	<i>grams</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>per cent</i>	<i>per cent</i>
Concord grape juice	50.0	125.0	129.7	147.9	0.040
				126.7	144.4		
				125.2	142.7		
				Average	145.0		
Synthetic apple jelly prepared in laboratory	17.5	125.0	None	112.7	128.5	0.714	0.731
				111.6	127.2		
				112.2	127.9		
				Average	127.9		
Commercial grape essence	10.0	125.0	112.9	128.7	0.040
				112.0	127.7		
				114.6	130.6		
				Average	129.0		
Commercial grape aroma essence	20.0	125.0	138.6	158.0	0.163
				138.6	158.0		
				137.6	156.9		
				Average	157.6		
Pectin concentrate	25.0	125.0	115.0	131.1	0.012
				109.2	124.5		
				112.6	128.4		
				Average	128.0		
Tomato juice	51.3 (50 cc.)	None	183.4	209.1	0.409
				185.7	211.7		
				182.5	208.1		
				Average	209.6		

¹ *This Journal*, 1927, 10: 264.

TABLE 5.—Continued.

Results obtained on fruit and fruit products when proposed method was used.

MATERIAL	WEIGHT	CITRIC ACID PRESENT	CITRIC ACID ADDED IN FINAL DETERMINATION	CITRIC ACID DETERMINED a	CITRIC ACID CORRECTED 1.14a	CITRIC ACID	
						Present	Found
	<i>grams</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>per cent</i>	<i>per cent</i>
Grape fruit juice	13.05 (12.5 cc.)	None	170.2	194.0	1.465
				165.6	188.8		
				167.7	191.2		
				Average	191.3		
Commercial gelatin dessert powder	7.5	None	119.4	136.1	1.775
				114.1	130.1		
				116.8	133.2		
				Average	133.1		
Gelatin dessert powder prepared in laboratory	12.5	135.0	None	115.4	131.6	1.080	1.049
				114.1	130.1		
				115.5	131.7		
				Average	131.1		
Currant jelly	17.5	None	79.1	90.2	0.508
				76.2	86.9		
				78.4	89.4		
				Average	88.8		
Apple jam prepared in laboratory	17.5	125.0	None	109.4	124.7	0.714	0.708
				107.2	122.2		
				109.6	124.9		
				Average	123.9		
Blackberry jam prepared in laboratory	17.5	125.0	None	110.0	125.4	0.714	0.712
				108.5	123.7		
				109.6	124.9		
				Average	124.7		
Raspberry jam	17.5	None	91.5	104.3	0.592
				92.1	105.0		
				89.1	101.6		
				Average	103.6		
Strawberry fountain sirup	21.0	60.0	80.4	91.7	0.146
				78.8	89.8		
				79.6	90.7		
				Average	90.7		
Pineapple fountain sirup	21.0	60.0	85.6	97.6	0.186
				87.3	99.5		
				87.7	100.0		
				Average	99.0		
Root beer prepared in laboratory	52.3	135.0	114.6	130.6
				114.9	131.0		
				116.7	133.0		
				Average	131.5		
Sucrose amaranth (1.2%) citric acid mixture prepared in laboratory	12.8	135.0	None	114.9	131.0	1.055	1.030
				115.9	132.1		
				116.0	132.2		
				Average	131.8		

tion may seem superfluous, attention is directed to the fact that in this table columns 2 and 3 pertain to the weight of material and citric acid in 100 cc. of prepared solution. In some instances the sample was measured, and then the weight was calculated from the gravity. With the exception of the five samples prepared in the laboratory, the materials originally contained citric acid. Not one of the materials used in the preparation of the products examined seemed to interfere with the determinations. Column 4 expresses the quantities of citric acid added to meet the requirements of the method.

It is interesting to note that the results for Concord grape juice indicate the presence of a small quantity of citric acid, corroborating Nelson's¹ work on the acids contained in Concord grapes. Attention is also directed to the small excess quantity of citric acid found in the synthetic apple jelly, due, no doubt, to the use in its preparation of the commercial grape essence, which is also included in Table 5. Commercial grape aroma essence shows 0.163 per cent of citric acid, which, it is quite likely, was added in the process of manufacture. At first trouble was experienced with the slow filtrations of the pectin concentrate because the large amount of pectin was not removed. It was found necessary to supplement the alkali and acid treatment by heating on the steam bath, as given under "Preparation of Solution". The tomato and grape fruit juices show larger quantities of citric acid than were indicated by titration (0.309 and 1.140 per cent), which would indicate that substantial quantities are present in these products in the combined form.

¹ *J. Am. Chem. Soc.*, 1925, 47: 1177.



HARRY SNYDER, 1867—1927.

HARRY SNYDER

Harry Snyder was born in Cherry Valley, New York, January 26, 1867, the son of David W. and Mary Harter Snyder. His ancestors settled in the Mohawk Valley in 1723. Members of the family fought in the French and Indian Wars, the American Revolution and the War of 1812.

As a boy, Harry Snyder attended country school at Saltspringville, graded school at Herkimer, New York, and Clinton Liberal Institute at Fort Plain. He then entered Cornell University, from which he was graduated with a Bachelor of Science degree in 1889 with honors in chemistry. During his junior and senior years he was an assistant to Dr. George C. Caldwell, head of the chemistry department of Cornell University. Following graduation he was instructor in Cornell University and assistant chemist in the Agricultural Experiment Station at Ithaca, New York.

He came to Minnesota as chemist of the Agricultural Experiment Station in 1891. In 1892 he was appointed Professor of Agricultural Chemistry in the University of Minnesota, and in 1907 he became Professor of Agricultural Chemistry and Soils. Professor Snyder served in this dual capacity until 1909, when he severed his connection with the University to become chemist of the Russell-Miller Milling Company of Minneapolis, Minnesota. He continued in the latter capacity until his death on October 11, 1927.

Mr. Snyder became active in the affairs of the Association of Official Agricultural Chemists shortly after he was appointed to the position of chemist of the Minnesota Agricultural Experiment Station. Thus, in the proceedings of the convention in 1892, there appears reference to a contribution which he had made to the study of certain analytical methods. He was fairly regular in his attendance at A. O. A. C. meetings, and contributed frequently to the study of both soils and food methods. He served as associate referee in 1905-6, and presented a report on the separation of the vegetable proteins. He was elected vice-president of the A. O. A. C. on November 16, 1906, and in addition to the duties of this office, he continued as associate referee on vegetable proteids and presented a report of this work at the Norfolk convention in October, 1907. On the occasion of this convention he was elevated to the presidency of the A. O. A. C. for the year 1908. His presidential address, read before the 25th Annual Convention at Washington, D. C., on November 13, 1908, was entitled "The Training of the Agricultural Chemist".

During his connection with the Agricultural Experiment Station, Professor Snyder contributed to the literature of agricultural chemistry in diverse ways. He published no less than 27 agricultural experiment station bulletins. The subjects of these publications covered a wide range of interests including soils, plant nutrition, the composition of various animal feeds, milling and baking, and human nutrition. During a part of this period, he served as collaborator of the United States Department of Agriculture in human nutrition researches. His studies on the digestibility and nutritive value of bread and macaroni, published during the period of 1899-1905, received wide recognition as the most thorough and adequate researches in this field. A number of journal papers were con-

tributed by him during his connection with the University of Minnesota and he served on the staff of technical writers of the *Encyclopedia Britannica*.

Professor Snyder was also the author of four textbooks published under the titles, "The Chemistry of Plant and Animal Life", "Dairy Chemistry", "Soils and Fertilizers", "Human Foods and Their Nutritive Value". These were organized originally for use in his classes in the School of Agriculture of the University of Minnesota.

The writer first made the acquaintance of Professor Snyder while he was instructing in the courses in chemistry in the School of Agriculture at Minnesota, and was greatly attracted by his genial personality as well as his manifest capabilities. Those who have attended agricultural colleges are aware that chemistry is one of the subjects not uniformly regarded with favor by the students; yet, it can be said that those of us who sat under Professor Snyder's instruction found that he possessed the happy faculty of maintaining our interest in the subject and of encouraging us to put our best efforts into this work. In conducting his classes Professor Snyder was never didactic; he always emphasized the fact that the science of agricultural chemistry was still in the making, and his classes came to recognize that he was bending his best efforts to advance their knowledge of this phase of science.

His capabilities were recognized by his confreres in the form of election to the presidency of the A. O. A. C., and he was honored in other connections. He was a fellow of the American Association for the Advancement of Science, a member of the Society for the Promotion of Agricultural Science, of the American Chemical Society, and of the Society of Sigma Xi.

Following his resignation from the faculty of the University of Minnesota, Mr. Snyder became chief chemist of the Russell-Miller Milling Company of Minneapolis in 1909. He maintained a keen interest in milling chemistry throughout the remainder of his life, and was a frequent contributor to the literature of this subject. His researches, and the papers based thereon, undoubtedly had a profound effect upon the development and revision of the definitions and standards for wheat flour. He appeared before many scientific and industrial organizations and always received the respectful hearing that was warranted by the precision of his work and the soundness of his judgment. He frequently represented not alone the firm with which he was connected, but also the Millers National Federation, which he served as an unofficial counselor and advisor.

Harry Snyder was married in 1890 to Adelaide Craig, the daughter of Dr. Austin Craig, clergyman and educator of New York, formerly President of Antioch College, and Adelaide Churchill Craig, a graduate of Antioch College. He is survived by Mrs. Snyder and two brothers. His untimely passing is mourned by many friends among his industrial and professional associates.

C. H. BAILEY.

FIRST DAY.

MONDAY—AFTERNOON SESSION—Continued.

No report on dairy products was given since no general referee was appointed following the death of Julius Hortvet.

REPORT ON BUTTER.

By LLOYD C. MITCHELL¹ (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.²), *Associate Referee*.

To determine the composition of butter, the three following separate and distinct operations are necessary at the present time: (A) sampling, (B) preparation of the sample for analysis, and (C) analysis. Since each of these operations may be, and often is, done by different persons, more than ordinary precautions are necessary in order to prevent the loss of moisture in each operation.

This year a collaborative study was made of the sampling of tub butter and of the methods for the analysis of butter, and the work begun last year³ on the preparation of butter samples for analysis was continued.

The associate referee desires to express his thanks and appreciation to all those that took part in the collaborative work.

(A) *Sampling of Tub Butter.*

Purpose of Sampling: The purpose of the sampling is to determine as definitely as possible (1) the accuracy and the dependableness of the trier method of sampling tub butter by comparison with the mixed entire-tub sample; and (2) the homogeneity of tub butter and the variation in the homogeneity from tub to tub and churning to churning with a view to deciding the number, size, and location of samples necessary for evaluating the true composition of the entire contents.

Outline of Work: Two or more tubs from a churning should be sampled, and, if possible, several churnings of both salted and sweet butters that have been subjected to varying conditions, such as (a) freshly churned butter cooled sufficiently for cutting into prints, (b) butter that has been in storage (sharp freezer) for a short time (1-2 weeks) and for a long time (8-10 months, or even longer). Full information regarding the condition of the butter sampled should be included in the report. It is believed that when all the results are tabulated, the work will give the normal variations to be expected in tub to tub and churning to churning of butter. Make duplicate determinations of moisture, non-fat solids, and fat (by difference). If time is limited, make only the moisture test.

Method for Experimental Sampling: CAUTION: Use every precaution to prevent a loss of moisture from exposure of the butter to the atmosphere.

¹ Presented by S. Alfend.

² Present address: Food, Drug and Insecticide Administration, Chicago, Ill.

³ *This Journal*, 1927, 10: 290.

1. Transfer the contents of the entire tub to a closed, moisture-tight receptacle suitable for mixing.

2. Divide the surface of the butter into three zones by concentric circles whose radii are in the ratio 1 : 2 : 3, and designate as inner, middle, and outer zones.

3. For butter held at a temperature suitable for cutting into prints, use the cold trier. For butter held in cold storage (sharp freezer), use the trier after heating by passing it several times through an alcohol flame.

4. Draw six samples by means of a trier inserted vertically and entirely through the butter, taking one core from the inner zone at a point two-thirds of the distance from the center to the edge of the zone, two cores at points equidistant from one another and slightly more than half the distance from the inner to the outer peripheries of the middle zone, and three cores at points equidistant from one another and slightly more than half of the distance from the inner and outer peripheries of the outer zone.

5. Place each core thus taken into separate, moisture-tight, half-pint containers, carefully transferring any water or brine adhering to any part of the trier to the sample container.

6. Soften the entire tub of butter in the closed moisture-tight receptacle so that when stirred or shaken the product will reach a temperature of 33°C. (A variation of 1° or 2° may not matter.)

7. If possible, mix the softened butter either (a) by stirring by means of a motor-driven "pull and push" mixer, or (b) by shaking by means of a motor-driven shaking apparatus. Otherwise, use the disc-stirrer commonly employed in mixing cream or milk in 10 gallon cans.

8. When properly mixed and while pouring the butter back into the tub, at equi-intervals take for analysis at least three samples, approximately of the same quantity as one core, in half-pint moisture-tight containers.

Notes on the Method of Sampling: 1. To prevent the loss of moisture due to evaporation, drainage, absorption, etc., particularly during the sampling, transferring, tempering, and mixing operations, it is suggested that the butter, preferably the 60-pound tub size, be transferred at the beginning of the experiment to a receptacle suitable for mixing. The receptacle should be of the same size and shape as a 60-pound butter tub, but with sufficient head-space to allow proper mixing. It should have a moisture-tight cover. The material used to make the receptacle should neither permit the absorption of nor be attacked by any of the ingredients of the butter.

2. Owing to possible segregation or gradation of the moisture or brine content from edge to center of a tub of butter, careful attention should be paid to the position of the cores, so that the average of the samples taken will approach as nearly as possible the true average of a tub of butter. If the surface of the butter is divided into three zones—inner, middle, and outer—by concentric circles whose radii are in the ratio 1 : 2 : 3, each zone would contain one-ninth, one-third, and five-ninths, respectively, of the total area. The volumes of the cylinders formed by the continuation downward of these peripheries would be in the same proportions. One vertical core in the inner zone, two in the middle zone, and three in the outer zone would contain one-sixth, one-third, and one-half, respectively, of the total sample taken. Thus, six samples taken from the three zones suggested in the method approaches a representative sample based on theoretical considerations.

3. The heated trier is used on frozen butter in order to eliminate the time necessary to bring the butter to a suitable condition for sampling with the cold trier. The "auger" method for sampling butter has been proposed. This method, applicable to frozen butter only, specifies boring into the hard butter with a power-drill and a three-fourths inch to one-inch bit. The borings are carefully transferred to the sample container with a spoon, and the drill-holes are plugged with cork-stoppers to prevent any borings

from dropping into the holes. It is hoped that some of the collaborators will compare the "auger" method of sampling with the trier methods. This would necessitate the sampling of additional tubs because all comparisons should be made against the mixed entire-tub samples.

4. If the claim of some butter-makers—that the water or brine is forced inward when butter is frozen—be true, then a sample of butter taken by inserting the trier diagonally through the butter could not be representative, because one-third of the sample would be from the inner zone, which represents only one-ninth of the butter. However, it is difficult to demonstrate this fact because the diagonal core would cut into at least one vertical core and possibly several cores and the error introduced by drainage has been found to be considerable. The location of each core within the zones obtained by application of simple methods of integral calculus, was chosen from theoretical consideration based on the assumption of a uniform gradation of moisture or brine content from edge to center.

5. Uniform sample containers are suggested in order to eliminate as far as possible any variation which may possibly be due to the size of the container used. Preliminary work indicates the advisability of transferring carefully to the sample container any water or brine adhering to the trier.

6. See Note 3 under "Proposed Method for the Preparation of the Sample" for explanation.

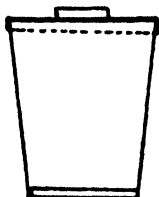
7. A convenient type of power-driven "pull and push" mixer is the "Hy-Speed" Mixer Type No. 4, manufactured by Alsop Engineering Company, New York, N. Y.

8. To ascertain the homogeneity of the entire tub of butter, take at least three samples.

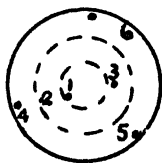
Two reports on the sampling of tub butter were received, one from M. M. Simpson, Chief Chemist, The Fairmount Creamery Company, Omaha, Nebr., and the other from Carl B. Stone, U. S. Food, Drug and Insecticide Administration, Minneapolis, Minn. Table 1 shows the individual results obtained by the collaborators.

COMMENTS ON SAMPLING OF TUB BUTTER BY COLLABORATORS.

M. M. Simpson.—The butter was removed from the tub by inverting it upon the lid. The liner was next removed and the container, made of extra heavy tinned copper the shape of a butter tub, but three and a half inches taller, was inverted over the butter and then turned upright. The parchment and cloth circles were removed, and the trier samples were taken from the locations indicated in drawing No. 1. Entire plugs



NO 2



NO. 1

were taken from top to bottom of the tub. A circle of sheet rubber was placed over the top of the can, and the lid, which was made of the same material as the container, was slipped over the outside, as illustrated in cut No. 2. The entire tub was then softened to such a temperature that upon stirring the butter reached the temperature indicated. An electric cream stirrer was used because it works like a malted milk

mixer. When thoroughly stirred, the butter was poured into a second container, and three samples were taken at equi-intervals during the pouring. All trier samples were placed in half-pint Mason jars with rubber rings in place. These samples were allowed to soften slowly to a temperature of 25°–28°C. with as little oiling off as possible. The jars were shaken before being opened and stirred with a spatula to a uniform consistency. Portions for analysis were removed from the tub samples immediately after attaining the temperature to which the butter was softened. Two grams was weighed in 2-inch

TABLE
Results on the

KIND OF BUTTER AND STORAGE PERIOD	CHURN* AND TUB NUMBER	BEFORE MIXING (TRIER SAMPLES)						
		MOISTURE			NON-FAT SOLIDS			
		Inner Zone	Middle Zone	Outer Zone	Inner Zone	Middle Zone	Outer Zone	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Sweet, 5 months in sharp freezer	A-1	15.11	14.95 14.67	15.08 14.99 15.31	1.06	0.62 0.64	1.11 0.72 1.01	
		A-2	14.83	14.93 14.78	14.93 14.91 14.92	1.10	1.03 1.04	1.09 1.12 1.06
			B-1	14.42	14.41 14.18	14.22 14.40 14.56	1.10	1.06 0.91
	B-2	14.37		14.53 14.58	14.28 14.38 14.68	0.84	0.85 0.92	0.90 0.82 1.02
		Sweet, 1 day in cooler	C-1	15.83	15.71 15.72	15.81 15.99 15.75	0.90	1.12 1.00
C-2	15.84			15.69 15.99	15.49 15.74 16.10	0.97	0.78 0.91	0.96 0.80 0.83
	D-1		16.28	16.10 16.34	16.16 16.14 16.19	0.91	0.95 0.93	0.91 0.86 0.87
D-2			16.15	15.98 16.37	16.10 16.48 15.88	1.01	1.00 0.99	0.96 0.93 0.95
	Sweet, 2 weeks in sharp freezer		E-1	16.11	16.28 16.53	16.24 16.37 16.42	0.78	0.81 0.77
E-2		16.30		16.69 16.01	16.76 15.80 16.43	0.78	0.88 0.93	0.90 0.91 0.88
		F-1	16.43	16.47 16.60	16.27 16.30 16.36	0.92	0.96 0.95	0.95 0.94 1.06
F-2			16.15	16.38 16.41	16.31 16.35 16.10	0.92	0.93 1.01	0.87 0.91 0.94
		Salted, 2 weeks in sharp freezer	G-1	15.13	14.73 15.08	15.46 15.14 15.50	3.53	3.11 3.29
G-2	14.71			14.85 14.76	15.21 15.31 15.11	3.45	3.51 3.39	3.70 3.66 3.72
	H-1		14.73	14.55 14.33	15.66 15.25 15.45	3.82	3.70 3.63	4.02 3.79 4.08
H-2			14.20	13.96 14.02	14.65 14.31 14.50	3.62	3.56 3.66	3.77 3.46 3.72
	Salted		Z-1	16.21 16.18	16.03 15.91 15.90 16.00	15.82 15.77 15.98 15.97 15.97 15.99	4.35 4.34	4.30 4.31 4.31 4.26
Z-2		15.63 15.58		15.64 15.57 15.74 15.66	15.70 15.67 15.61 15.68 15.71 15.61	3.99 4.09	4.10 4.04 4.14 4.05	4.11 4.07 4.02 4.09 4.07 4.02

1.

sampling of tub butter.

FAT (BY DIFFERENCE)			AFTER MIXING (MIXED SAMPLES)			
Inner Zone	Middle Zone	Outer Zone	Temperature when mixed	Moisture	Non-fat Solids	Fat (by difference)
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>°C.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
83.83	84.43	83.81	32-34	14.90	1.11	83.99
	84.69	84.29		14.87	0.87	84.26
		83.68		14.94	0.87	84.19
84.07	84.04	83.98	32-34	15.01	1.01	83.98
	84.18	83.97		15.03	1.12	83.85
		84.02		15.06	1.03	83.91
84.48	84.53	84.82	32-34	14.36	0.84	84.80
	84.91	84.66		14.34	0.97	84.69
		84.43		14.36	1.07	84.57
84.79	84.62	84.82	32-34	14.30	1.00	84.70
	84.50	84.80		14.32	0.94	84.74
		84.30		14.40	0.97	84.63
83.27	83.17	83.20	32-34	16.16	0.95	82.89
	83.28	83.20		16.20	0.85	82.95
		83.34		16.23	0.91	82.86
83.19	83.53	83.55	32-34	16.16	0.91	82.93
	83.10	83.46		16.18	1.06	82.76
		83.07		16.09	0.83	83.08
82.81	82.95	82.93	26-28	16.23	0.91	82.86
	82.73	83.00		16.23	0.87	82.90
		82.94		16.20	0.85	82.95
82.84	83.02	82.94	26-28	16.19	0.94	82.87
	82.64	82.59		16.07	0.96	82.97
		83.17		16.20	0.95	82.85
83.11	82.91	83.01	26-28	16.24	0.85	82.91
	82.70	82.80		16.33	0.88	82.79
		82.77		16.31	0.79	82.90
82.92	82.43	82.34	26-28	16.42	0.82	82.76
	83.06	83.29		16.41	0.87	82.72
		82.69		16.39	0.83	82.78
82.65	82.57	82.78	26-28	16.35	0.84	82.81
	82.45	82.76		16.31	0.85	82.84
		82.58		16.33	0.86	82.81
82.93	82.69	82.82	26-28	16.18	0.89	82.93
	82.58	82.74		16.16	0.87	82.97
		82.96		16.11	0.88	83.01
81.34	82.16	81.17	26-28	15.27	3.22	81.51
	81.63	81.80		15.28	3.15	81.57
		81.15		15.28	3.15	81.57
81.84	81.64	81.09	26-28	15.13	3.60	81.27
	81.85	81.03		15.12	3.64	81.24
		81.17		15.13	3.56	81.31
81.45	81.75	80.32	26-28	15.48	4.11	80.41
	82.04	80.96		15.51	4.05	80.44
		80.47		15.48	4.17	80.35
82.18	82.48	81.58	26-28	14.68	3.75	81.57
	82.32	82.23		14.66	3.70	81.64
		81.78		14.65	3.69	81.66
79.44	79.67	80.02		15.86	4.23	79.91
79.48	79.78	79.96		15.87	4.22	79.91
	79.79	79.67		15.99	4.26	79.75
	79.74	79.77		15.98	4.31	79.71
		79.67		15.98	4.30	79.72
		79.69		15.92	4.28	79.80
80.38	80.26	80.19		15.76	3.98	80.26
80.33	80.39	80.26		15.73	4.02	80.25
	80.12	80.37		15.86	3.95	80.19
	80.29	80.23		15.85	3.97	80.18
		80.22		15.84	3.97	80.19
		80.37		15.83	4.00	80.17

by $\frac{1}{4}$ -inch aluminum dishes and dried in a water-jacketed oven. The dried samples were washed with ether into porcelain Gooch crucibles having thin mats of asbestos to retain the non-fat solids. The trier and plugs pulled from both tubs of churning No. H, the last two tested, showed an extra large quantity of free water. This free moisture was transferred to the sample jar, that is as much of it as it was possible to collect. Both of these tubs showed the greatest variation between trier and tub samples. The two tubs of sweet butter from churning No. C showed a corresponding variation of trier and tub, but no notation was made as to whether or not there was considerable water. Most of the trier and plugs pulled dry. The variation seems to be greater from churning to churning than from tub to tub. A relationship seems to exist between the variation of the averages of the trier samples and tub samples and the amount of free moisture on the trier.

Carl B. Stone.—Two tubs of one churning were sampled by the cold-trier method. Six samples were taken from each tub by the method of circles proposed by the associate referee, and three samples were taken from the melted tub in each case. All the samples were analyzed by the official method. The fat was determined by the official indirect method. Samples were heated 3 hours at the temperature of boiling water for moisture determination. All the samples from both tubs were placed in half-pint Mason fruit jars. Samples were then heated in order to soften the butter (30° – 38° C.) and thoroughly shaken in order to secure a uniform sample. The lid was removed from each jar, and the sample was stirred with a spoon before being weighed. Because a larger container was not available, $13\frac{1}{2}$ pounds of butter was cut off the top of tub No. 1, and 49.5 pounds of butter was placed in the metal container and heated by the aid of steam. When the butter was partly melted, it was stirred by the aid of a large cream stirrer until a uniform consistency was secured. As the butter was poured back into the tub, three samples were taken at equidistant points and placed in one-half pint Mason jars. At this time the temperature of the butter was 30° C. Less than 1 ounce of moisture was lost in heating the 49.5 pounds of butter, and this was not included in the results as reported. Tub No. 2 was left in the laboratory ice-box for 42 hours before it could be sampled. The entire tub contents, after the trier samples had been taken, were placed in the metal container to be melted. Loss of moisture could not be calculated as part of the butter was lost in the mixing. Only $4\frac{1}{2}$ inches of clearance remained when the butter was melted in the metal container.

C. S. Brinton, Chief, U. S. Food, Drug and Insecticide Administration, Philadelphia, Pa., submitted data relative to the sampling of salted tub butter. All the tubs from three churnings were sampled, and the butter was analyzed separately by A. M. Henry and S. C. Rowe. Each sample consisted of three cores of butter from a tub and was taken by inserting a trier vertically and entirely through the butter at points two-thirds of the distance from the center to the edge of the tub. Later composite samples consisting of three tubs each were drawn in a similar manner from each churning. The samples were prepared by the mechanical stirrer method and then stirred with a spoon immediately before being weighed. The official method was used for the analysis. The results of the sampling are shown in Table 2.

Six churnings of salted butter were sampled and analyzed by the Minneapolis Station of the U. S. Food, Drug and Insecticide Administration; they were then shipped to New York City where the churnings

TABLE 2.
Results on sampling tub butter (Brinton).

CHURN NUMBER	NUMBER OF TUBS IN CHURNING	TOTAL VARIATION IN CHURNING		RESULTS FOR THREE TUBS ANALYZED			
				Separately (average of 3 tubs)		Compositely	
		Moisture	Fat	Moisture	Fat	Moisture	Fat
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	5	1.38	1.19	15.65	80.24	15.65	80.24
2	12	0.63	1.13	15.39	80.47	15.39	80.47
3	13	3.32	2.61	16.90	79.10	16.49	79.42

were again sampled and analyzed by the New York Station of the U. S. Food, Drug and Insecticide Administration. The cores were taken by inserting a trier vertically and entirely through the butter at a point two-thirds of the distance from the center to the edge of the tub. Each sample for analysis consisted of three cores thus taken, one from each of three tubs in a churning, but the New York samples were not taken from the same tubs of the churnings as the Minneapolis samples. The samples were prepared as stated previously. The Minneapolis Station used the official method of analysis, while the New York Station used the proposed method for the first four churnings and the official method for the other two. The results of this work are given in Table 3.

TABLE 3.
Results on sampling tub butter (Minneapolis and New York Stations).

CHURN NUMBER	MINNEAPOLIS STATION			NEW YORK STATION		
	Moisture	Non-fat Solids	Fat (by difference)	Moisture	Non-fat Solids	Fat (by difference)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	16.30	3.27	80.43	16.35*	3.42*	80.23*
2	15.16	3.79	81.05	15.00*	3.81*	81.19*
3	15.07	2.47	82.46	16.60*	2.86*	80.54*
4	15.55	2.17	82.28	15.51*	2.56*	81.93*
5	18.94	2.66	78.40	18.96	2.53	78.51
6	18.76	3.28	77.96	18.60	3.48	77.92

* By proposed method; all others by official method.

DISCUSSION.

The results given in Table 1 fail to disclose any appreciable loss in moisture in sampling tub butter by means of a trier. This is not in

conformity, however, with conclusions drawn from a large amount of previous work¹. It is noted that no mention is made of the shape or size of the triers used. This is probably an important consideration, as indicated by the work of Ellenberger and Newlander, and deserves further investigation. The work of Simpson and Stone gives no definite indications of any regular segregation of moisture during freezing.

The maximum variations among the six samples from a single tub and the surprisingly small differences between two tubs in the same churning indicate that the collaborators sampled churnings of butter which had been well worked and was quite uniform in composition. Previous work in the associate referee's laboratory on salted butter led him to expect a greater variation in the tubs that had been stored for some months in a sharp freezer and to conclude that sweet butter will usually be more uniform in composition than will salted butter and will remain more uniform during storage in a sharp freezer. Of the eight churnings sampled by Simpson, only two were of salted butter, and it is significant that one of these two showed the greatest variation within a single tub and the greatest difference between the average results of two tubs in the same churning.

By taking three cores from a tub, Henry and Rowe were able to secure samples of butter which yielded excellent checks when the average results on samples from three individual tubs were compared with the results on composite samples from the same three tubs, the samples being taken at different times. One churning showed a moisture variation of 0.41 per cent and a fat variation of 0.32 per cent between the averages on the samples from the three tubs analyzed separately and on the samples from the same three tubs analyzed compositely. The maximum variations among the three tubs that were analyzed separately and the average results compared with the results from a composite sample from the same three tubs were as follows: for moisture, 0.35, 1.36, and 0.93 per cent, respectively; and for fat, 0.84, 1.19, and 1.11 per cent, respectively.

The work done at the Minneapolis and New York Stations checked well within the limit of error, with the exception of churning number three. The samples consisted of one core from each tub from two lots of three tubs each in the same churning. No notation was made as to whether the cores and trier pulled wet or dry.

From the limited number of tubs sampled, it appears that six cores taken from a tub of sweet butter by means of a trier in the manner outlined in the directions submitted to the collaborators will give a representative sample. The associate referee believes that this conclusion may be warranted, provided the condition of the butter is such that the

¹ Ellenberger and Guthrie. *J. Dairy Sci.*, 1925, 8: 80; Smith, Mitchell and Alfend. Unpublished report, April, 1926; Ellenberger and Newlander. *Vermont Agr. Expt. Sta. Bull.* 265, March, 1927.

trier and cores pull dry. On the other hand, if the shape of the trier or the condition of the butter is such that the trier and core have drops of water or brine adhering to them, even though practically all the adhering water or brine is transferred to the sample container along with the core of butter, the six cores will often give a sample containing less moisture than a sample from the mixed entire tub of butter. The variations in salted butter are usually wider than in sweet butter, as shown by the results of the collaborators, and they are often even wider than these results show. The data obtained on sampling tub butter by means of a trier, in the opinion of the associate referee, are too few to warrant definite conclusions at this time, and more work must be done, especially with salted butter, with reference to the shape of the trier.

RECOMMENDATIONS¹.

It is recommended—

(1) That the method for sampling print butter given in the 1926 report be adopted as official.

(2) That the method for sampling tub or bulk butter given in the 1926 report be continued as tentative. (Note.—Sentence two under recommendation (5) should read: The three trierfuls may be taken from a single tub, or one trierful may be taken from each of three tubs from the same churning.)

(B) Preparation of Sample.

Outline of Work: In order to ascertain the probable loss of moisture in samples prepared in both open and closed containers, each sample should be reprepared by the same method, and separate one-half pounds of butter in moisture-tight containers should be used for each experiment. It would be highly desirable to use "synthetic" butter of known composition provided a mixture could be obtained which had the consistency and physical structure of natural butter. (The associate referee has been unable to prepare a "synthetic" butter consisting of butter fat, water, salt, and dried powdered defatted skimmed milk which has the consistency and physical structure of butter.) Make duplicate determinations of moisture, non-fat solids, and fat (by difference). If time is limited, make only the moisture test.

Since the first collaborative study of the present official method², made during 1926³, showed the method to be indefinite, it seemed desirable for the associate referee to outline a method so clearly that uniformity in the results would be obtained. After reviewing the shaking and mixing modifications of the official method reported last year by the collaborators, the associate referee outlined the following directions, adhering as closely as possible to the present official method.

Shaking Modification of Official Method.

Soften the entire sample in a closed vessel, warming slowly at 40°–45°C. Shake frequently until all lumps have disappeared, using care that the ingredients do not be-

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 75.

² *Methods of Analysis*, A. O. A. C., 1925, 276, 67.

³ *This Journal*, 1927, 10: 290.

come separated. Continue to shake vigorously without cooling other than in the air until a perfectly homogeneous, soft, pasty mass is obtained. Weigh the portions for analysis at once.

Mixing Modification of Official Method.

Allow the entire sample to stand in a closed vessel at room temperature (25°–28°C.) overnight, or in an incubator at 25°–28°C. until the butter mass reaches the same temperature. Then mix vigorously with a spatula until a perfectly homogeneous, soft, pasty mass is obtained, and the butter assumes a creamy consistency. Weigh the portions for analysis at once.

Proposed Mechanical Stirrer Method.

1. Soften the sample, 250–500 grams, in a closed vessel to such an extent that on stirring for 2–3 minutes the product will reach a temperature of 31°–34°C.

2. Stir with a malted milk stirrer for 2–3 minutes with an up-and-down movement of the stirring device, at the same time slowly moving the vessel horizontally so that the stirrer reaches all parts of the sample.

3. The final temperature must be 31°–34°C.

4. If the temperature is below 31°C., continue the softening and stirring until this temperature is reached. A temperature above 34°C. will indicate usually that the sample has been softened too much, and is likely to separate. In this case, cool the sample until solid and repeat the softening and mixing.

5. Weigh the portion for analysis within 3–4 hours with a room temperature of approximately 25°C., and within one-half hour with a room temperature of 28°C. or above.

6. Do not permit the butter to cool below 23°C. before weighing the portions for analysis.

NOTES ON PROPOSED STIRRER METHOD.

1. Containers should be approximately twice the size of the sample; that is, half-pound samples of butter should be placed into pint moisture-tight containers, one-pound samples in quart, moisture-tight containers, and smaller samples in proportionately smaller containers. Samples smaller than half-pound, or approximately 225 grams, cannot be prepared satisfactorily by means of a stirrer. The temperature of the butter mass should be such that the stirring will bring it up about 1°C. to 32°–34°C. Experience will indicate to the analyst when a sample is sufficiently soft to be mixed properly.

2. A convenient mixer is Type No. 2, manufactured by the Hamilton Beach Manufacturing Co., Racine, Wis.

3. The principle of the proposed method is based on the consistency of the butter at the time it is mixed, which in turn depends on the condition or physical state of the butter fat, and the condition and amount of the solids other than fat. The optimum condition for a thoroughly homogeneous incorporation of all the complex constituents of the product is probably one in which the butter fat is at, or just above, its melting point. Attempt is made to define this condition by means of the thermometer. As the melting point of butter fat varies (28°–33°C.—abnormal butter and oleomargarine have greater variations in the melting point), it is likely that for different samples of butter the optimum temperature for mixing may also vary directly with the melting point. Some lots of butter have had to be stirred at 34°–35°C. to obtain the proper consistency, while others were far too fluid at 33°C. and had to be stirred at 31°–32°C. No samples of butter have been mixed, however, which showed a greater range in temperature than 31°–35°C. The maximum temperature of 34°C. is given in the method because many samples of butter would separate if mixed at a higher temperature.

4. The "melting point" of butter differs considerably from its "solidification or congealing point", the latter value being 19°–22°C. For this reason, it is always necessary to prepare the butter by softening the sample—never by cooling after the butter fat has been nearly or completely melted. Once the sample is overheated, it is necessary to cool the butter to about 22°C. or lower, to obtain a consistency comparable with the consistency of butter warmed up to 33°C. In case of overheating, the sample should be cooled to below 20°C. until solid, then softened to about 31°–33°C. before being mixed. Failure to cool the butter until solid will always cause separation. One or two samples have been examined even when this treatment failed to prevent separation after the sample had once been overheated.

5. It has been observed in the analysis of many hundreds of samples of butter prepared by the proposed stirrer method that samples properly prepared will invariably fail to show separation on 3–4 hours' standing at a room temperature of 25°C. Even with room temperature of 31°–35°C., at least two hours' standing is required before any separation occurs. When working at a room temperature of 28°–30°C., or above, as a precaution it is suggested that the prepared sample be set in a refrigerator for 20–30 minutes if analysis cannot be started within a half-hour after mixing, but the butter should not be allowed to go below a temperature of 23°C. With a room temperature of 25°C., no separation has been noticed on samples of butter which had stood for a week or more.

6. During the past year, it was found that the moisture or brine in prepared samples that were allowed to harden showed a tendency to segregate in that portion of the butter which was the last to become hard.

Reports on the preparation of the sample for analysis were received from H. W. Haynes, C. H. Hickey, and Ernest L. P. Treuthardt, U. S. Food, Drug and Insecticide Administration, Boston, Mass.; J. T. Keister, Food Control Laboratory, U. S. Food, Drug and Insecticide Administration, Washington, D. C.; W. D. Richardson, Swift & Co., Chicago, Ill.; C. A. Roach, U. S. Food, Drug and Insecticide Administration, Chicago, Ill.; and Carl B. Stone. The work of Haynes, Hickey, and Treuthardt is found in Table 4, and that of Richardson, Roach and Stone in Table 5.

COMMENTS ON PREPARATION OF SAMPLE BY COLLABORATORS.

Haynes, Hickey, and Treuthardt.—The official method, determining fat by difference, was used. To avoid the variation due to different subdivisions, the work was done on a single 2 pound roll of butter, which was cut into quarters marked A, B, C, and D. All three analysts worked on subdivision A, and each took one of the remaining subdivisions. Instead of preparing different subdivisions by different methods, each subdivision was prepared by each method in turn. According to Newlander and Ellenberger¹, the loss of moisture is least when prepared by the shaking method and greatest when prepared by the mechanical stirrer. With this information in mind, the analyst prepared each sample by the various methods in turn, as follows: (1) Shaking method, (2) shaking method, (3) mixing method, (4) mixing method, (5) mechanical stirrer, (6) mechanical stirrer, (7) shaking method. Portions were weighed out for analysis after each preparation. The samples were cooled in the refrigerator for one-half day after each sampling before the next preparation was made. By following the above series on the same sample, it is believed that a line may be obtained on the moisture

¹ Vermont Agr. Sta. Bull. 263.

TABLE 4.
Results on preparation of sample.
(Official method of analysis used.)

SAMPLE NUMBER	MOISTURE			NON-FAT SOLIDS			FAT (BY DIFFERENCE)		
	Haynes	Hickey	Treut-hardt	Haynes	Hickey	Treut-hardt	Haynes	Hickey	Treut-hardt
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
A 1*	17.65 17.70	17.65 17.65	17.76 17.72	2.58 2.48	2.50 2.58	2.48 2.47	79.77 79.82	79.85 79.77	79.76 79.81
A 2*	17.68 17.78	17.68 17.70	17.57 17.62	2.57 2.48	2.50 2.50	2.63 2.50	79.75 79.74	79.82 79.80	79.80 79.88
A 3†	17.39 17.43	17.48 17.50	17.47 17.41	2.47 2.46	2.48 2.48	2.45 2.38	80.14 80.11	80.04 80.02	80.08 80.21
A 4†	17.23 17.24	17.30 17.20	17.59 17.53	2.44 2.50	2.45 2.40	2.43 2.40	80.33 80.26	80.25 80.40	79.98 80.07
A 5‡	16.62 16.61	16.83 16.75	16.74 16.76	2.47 2.47	2.60 2.58	2.45 2.47	80.91 80.92	80.57 80.67	80.81 80.77
A 6‡	16.42 16.43	16.28 16.28	16.33 16.27	2.53 2.43	2.45 2.53	2.58 2.59	81.05 81.14	81.27 81.19	81.09 81.14
A 7*	16.01 16.20	16.18 16.20	16.22 16.18	2.46 2.38	2.48 2.40	2.52 2.49	81.53 81.42	81.34 81.40	81.26 81.33
Average variation: For each analyst For three analysts	0.06	0.03 0.20	0.05	0.06	0.05 0.13	0.04	0.05	0.07 0.24	0.07
Maximum variation: For each analyst For three analysts	0.19	0.10 0.39	0.06	0.10	0.08 0.16	0.13	0.11	0.15 0.42	0.13
	SUB. B	SUB. C	SUB. D	SUB. B	SUB. C	SUB. D	SUB. B	SUB. C	SUB. D
BCD 1*	17.68 17.68		17.70 17.66	2.48 2.48		2.47 2.50	79.84 79.84		79.83 79.84
BCD 2*	17.71 17.70	17.73 17.50	17.66 17.76	2.43 2.50	2.53 2.50	2.47 2.49	79.86 79.80	79.74 80.00	79.87 79.75
BCD 3†	17.53 17.53	17.50 17.50	17.56 17.53	2.52 2.58	2.55 2.58	2.45 2.43	79.95 79.89	79.95 79.92	79.99 80.04
BCD 4†	17.60 17.54	17.38 17.35	17.28 17.24	2.49 2.50	2.58 2.58	2.42 2.44	79.91 79.96	80.04 80.07	80.30 80.32
BCD 5‡	17.40 17.33	17.30 17.15	17.37 17.33	2.45 2.38	2.45 2.50	2.42 2.44	80.15 80.29	80.25 80.35	80.21 80.23
BCD 6‡	17.18 17.37	17.00 16.98	17.29 17.27	2.43 2.51	2.38 2.45	2.60 2.58	80.39 80.12	80.62 80.57	80.11 80.15
BCD 7*	17.26 17.23	17.18 17.10	17.18 17.28	2.44 2.44	2.50 2.58	2.47 2.39	80.30 80.33	80.32 80.32	80.35 80.33
Variation: Average Maximum	0.05 0.19	0.08 0.23	0.05 0.10	0.04 0.08	0.04 0.08	0.03 0.08	0.09 0.27	0.08 0.26	0.04 0.12

* Official—shaking modification.

† Official—mixing modification.

‡ Proposed mechanical stirrer.

TABLE 5.
Results on preparation of sample.
 (Official method of analysis used.)

COLLABORATOR	METHOD OF PREPARATION	SAMPLE NUMBER	MOISTURE	NON-FAT SOLIDS	FAT (BY DIFFERENCE)
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
W. D. Richardson	Official-shaking modification	1	16.67	4.08	79.25
			16.70	4.12	79.18
	Official-mixing modification	1	16.50	4.05	79.45
			16.52	4.05	79.43
	Proposed-mechanical stirrer	1	16.28	4.15	79.57
			16.30	4.12	79.58
C. A. Roach	Official-shaking modification	2	16.60	4.12	79.28
			16.60	4.10	79.30
	Official-mixing modification	2	16.20	4.05	79.75
			16.47	4.03	79.50
	Proposed-mechanical stirrer	2	16.13	4.15	79.72
			16.20	4.08	79.72
C. A. Roach	Official-shaking modification	1	15.68	2.50	81.82
			15.80	2.51	81.69
	Official-mixing modification	1	15.95	2.52	81.53
			15.93	2.50	81.57
C. B. Stone	Proposed-mechanical stirrer	1	15.63	2.45	81.92
			15.78	2.35	81.87
	Official-shaking modification	A 1	15.64	4.03	80.33
			15.56	4.10	80.34
	(Repeated)	A 2	15.63	4.03	80.34
			15.59	4.02	80.39
	Official-mixing modification	B 1	15.64	4.10	80.26
			15.66	4.01	80.33
	(Repeated)	B 2	15.64	4.08	80.28
			15.59	4.07	80.37
C. B. Stone	Proposed-mechanical stirrer	C 1	15.61	4.06	80.33
			15.65	4.10	80.25
	(Repeated)	C 2	15.57	3.96	80.47
			15.56	3.99	80.45

loss caused by the preparation and weighing-out without the error due to variation in different packages. The greater progressive decrease in moisture in subdivision A may be due in part to the fact that sampling by three analysts increased the exposure of the open jar to the air, and partly to the fact that half the sample was spilled during the first run. On warming the samples for the mechanical stirrer method considerable condensation of moisture occurred on the top of the jars. Attempt was made to incorporate this moisture into the butter. The final temperature prescribed in the mechanical stirrer method is higher than we think desirable. In past work we have preferred a

final temperature not over 31°C., which gave a sample of somewhat more pasty consistency than is possible at a higher temperature. After completing the mechanical stirring it had also been our practice to give a final stirring with a spatula to ensure that all portions of the sample are mixed, there being a tendency for melted portions to remain in the depression around the bottom of the jar.

Keister.—(Preparation and analysis of the sample.) The temperatures of the mechanically mixed subdivisions were 32, 31, and 31°C., respectively, of "A", "B", and "C". There being but two subdivisions of Sample A, subdivision A1 was mechanically mixed after the first analysis was finished. Seven salt determinations were made on Sample C, one or more from each subdivision, and the average percentage of sodium chloride found was 1.96 (range 1.90–2.07). The fat figures by the indirect official method correspond more closely with those obtained by the direct official method than with those by the proposed method. The moisture figures by the proposed method are uniformly lower than those by the official method—usually 0.20–0.30 per cent difference. Taking the figures on Sample C, which represent direct determinations (except curd by difference), we find the following totals: Shaking modification—100.05 per cent, mixing modification—99.92 per cent, mechanical stirrer—99.955 per cent, and shaking subdivision remixed—100.01 per cent, which looks very favorable for the official method. In Samples B and C the mechanically mixed subdivisions show more closely agreeing fat results than those by the other two methods of mixing. In the shaking modification, the temperatures 40°–45°C., given in the directions, are too high; the temperatures 32°–35°C. are preferable and sufficiently high to avoid separation of fat and water. None of the three samples worked upon, in my opinion, would permit a temperature of 40°–45°C. without separation. The proposed method requires less time and gives good results, but the figures obtained for moisture when considered in the light of the other data (comparative fat results) appear to be low in some cases, indicating that all the moisture was not driven off in two hours' drying.

Richardson.—There apparently is a loss of moisture with increased temperature and agitation which is not encountered in the method of shaking in a closed vessel.

Roach.—The Official Method: Shaking and Cooling in the Air. When butter is heated to 40°C. (as per instructions) it is almost impossible to cool it in the air to such a temperature that it can be weighed properly. This is especially true during the warm months. I shook this sample in the air for 30 minutes and then finally cooled it under the tap.

Mechanical Stirrer Method: There is nothing gained in this method. It is somewhat slower than the spoon-mixing method. The lower results obtained by this method indicate that there is some loss in moisture during the operation.

Conclusion: The Spoon or Spatula Mixing Method is a time saver and requires no special apparatus. Moisture results are higher. I can see nothing against it.

Stone.—The regular official method of analysis was used on all samples. The fat was determined by the official indirect method. The three samples were prepared and reprepared according to the referee's directions. The three samples were taken from a larger batch of butter that was melted, thoroughly mixed, and placed in pint jars, and after being allowed to solidify in the ice-box, they were properly labeled and prepared for analysis by the three methods given in the directions. The only variation in the analysis might be noticed in the mechanical stirrer method. A loss of 0.06 per cent moisture was noted in the preparation of the sample for the two sets of analyses. This might be attributed to experimental error as it is a very close margin. I should like to suggest that in next year's work the following directions be followed: Soften the sample at a temperature of 30°–36°C., mix for 2 minutes with a mechanical stirrer, place lid on jar, and shake thoroughly. When the sample is to be weighed out, stir it again with a spoon and then weigh.

DISCUSSION.

It is evident from the data in Tables 4, 5, and 7 and the comments of the collaborators that no entirely satisfactory method for the preparation of butter samples for analysis has yet been devised. In general, the results indicate that any method of preparation which involves exposure of the butter to the air during such preparation is liable to error owing to the loss of moisture, this loss being somewhat greater in the case of the mechanical stirrer method, which uses a higher temperature, than in the case of the mixing modification of the official method, in which a spatula is ordinarily used for mixing the butter. In some instances there seemed to be no appreciable loss of moisture in the mechanical stirrer method, while in other instances there seemed to be a distinct loss of moisture encountered even in the shaking modification of the official method.

The shaking modification is presumably the safest because theoretically no moisture is lost during the preparation of the sample. However, this method involves other difficulties, among which are the following: (1) The room temperature is not high enough ordinarily to render the butter sufficiently soft for proper shaking and close attention must be given to warming or the butter will become melted and the ingredients will separate, which condition tends to increase the difficulties of a suitable preparation of the sample; (2) after the butter is sufficiently soft, it must be shaken vigorously until a perfectly homogeneous, semi-solid mass is obtained, but in warm weather the butter may not reach this state of semi-solid consistency unless cooled in some manner. If the butter is cooled sufficiently to harden or congeal, the homogeneity is apparently destroyed; (3) if no suitable mechanical shaking machine is available, the shaking must be done by hand, which is more or less objectionable; (4) it generally takes more time to prepare a sample properly by shaking than by stirring or mixing; (5) an uncertainty exists as to whether the sample has been properly mixed; (6) there is the necessity to weigh immediately for analysis when the sample will not thicken sufficiently at room temperature to permit standing without separation.

As in last year's work, it is seen that some careful, experienced analysts got remarkably good results by practically any method, whereas other analysts equally as careful and experienced failed to get satisfactory results by almost any method. The proponents of the several methods studied have in each case given many data to prove that their particular methods were accurate and correct, while other methods were at fault. The majority of the collaborators, however, failed to substantiate fully the claims of any of the proponents.

RECOMMENDATION FOR PREPARATION OF SAMPLE.

It is recommended—

(1) That the mixing modification be removed from the present official method for preparation of a butter sample.

(2) That a study be made of the shaking modification of the present official method with a view to determining the best conditions for the preparation of sample and of describing clearly these conditions in order to eliminate any uncertainties in the method.

(C) *Analysis of Sample.*

Outline of Work: Analyze one or more samples of butter by all the methods, making the determinations in duplicate, and report the method used. It would also be desirable to use "synthetic" butters.

Official Method.

Determine the moisture, non-fat solids, and fat by the official methods¹.

Proposed Method.

MOISTURE, FAT, AND SALT.

APPARATUS.

Specially prepared Gooch crucible.—Prepare a Gooch crucible of about 30 cc. capacity with a 0.1 gram pad of asbestos and place thereon 20 grams of R. R. alundum, 90 mesh. (This is a crystalline alumina especially prepared for carbon determinations. After use, the crucible is re-prepared for further use by igniting in a muffle, washing with water, and drying at 100°–105°C.)

DETERMINATION.

Weigh accurately in the weighed, specially prepared Gooch crucible 1.0–1.5 grams of the prepared sample, dry for 2 hours at 100°–105°C., cool, weigh, and calculate the percentage loss in weight as moisture. Extract the fat from the dried sample by placing the Gooch crucible in a closed-system extraction apparatus and extracting for 30–40 minutes with carbon tetrachloride. Adjust the heat so that the solvent drops into the crucible at the same rate as the crucible drains and keep the crucible nearly full of the solvent. When the extraction is complete, remove most of the solvent remaining in the crucible by applying suction for a few seconds. Dry the crucible for 30 minutes at 100°–105°C., cool, weigh, and calculate the percentage of non-fat solids. Calculate the percentage of fat by subtracting the sum of the percentages of moisture and non-fat solids from 100.

If it is desired to determine the salt, wash it out of the non-fat solids with water and titrate the aqueous solution with standard silver nitrate solution, using potassium chromate indicator.

Notes on Proposed Methods: Gooch crucibles prepared as described will retain approximately 10 per cent of the weight of the absorbent material used without loss of fat during the drying period. Only sufficient asbestos to hold the absorbent material should be used, as it appears to be impossible to wash all the fat out of the asbestos without using excessive quantities of solvent. The R. R. alundum, which is 90-mesh, crystalline alumina specially prepared for carbon determinations, can be purchased ready for use from the Norton Company, Worcester, Mass., for a nominal sum. It has been found that drying a sample of butter prepared by the proposed stirrer method

¹ *Methods of Analysis*, A. O. A. C., 1925, 276: 68, 69, 70.

for 2 hours and accepting the result as the minimum weight will give as close to the true value as will be obtained by drying at hourly intervals to the minimum weight. It does not appear to be necessary to cool the dried sample in a desiccator. A convenient closed-system extractor has been illustrated¹. By titrating the salt, the possible error due to the volatilization of the sodium chloride during ashing is eliminated.

Reports on the methods of analysis were received from H. W. Haynes, C. H. Hickey, and Ernest L. P. Treuthardt; J. T. Keister; W. D. Richardson; C. A. Roach; Howard R. Smith, U. S. Food, Drug and Insecticide Administration, Baltimore, Md.; and Carl B. Stone. The results of Haynes, Hickey, and Treuthardt are found in Table 6, and those of Keister, Richardson, Roach, Smith, and Stone in Table 7.

TABLE 6.

Results on analysis of samples

(Three analysts working on same samples and using the proposed mechanical stirrer method.)

COLLABORATOR	SAMPLE NUM- BER	MOISTURE		NON-FAT SOLIDS		FAT		
		Official	Proposed	Official	Proposed	Official		Proposed
						By dif- ference	Direct	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
H. W. Haynes	1	15.92 15.92	15.68	2.87 3.11	3.16	81.21 80.97	81.17 81.16	81.16
C. H. Hickey	1	15.83 15.78	15.86 15.85	3.00 3.15	3.20 3.15	81.17 81.07	81.83 79.42	80.94 81.00
E. L. P. Treuthardt	1	15.74 15.76	15.73 15.70	3.01 3.06	3.13 3.11	81.25 81.18	81.08 81.30	81.14 81.19
H. W. Haynes	2	13.22 13.24	13.08	3.13 3.30	3.18	83.65 83.46	83.62 83.64	83.74
C. H. Hickey	2	13.24 13.18	13.15 13.24	3.13 3.00	3.12 3.09	83.63 83.82	83.35 84.38	83.73 83.67
E. L. P. Treuthardt	2	13.07 13.15	13.04 12.99	3.01 3.00	3.11 3.12	83.92 83.85	83.47 84.10	83.85 83.89
Average		14.50	14.43	3.06	3.12	82.44	82.38	82.43
Variation in duplicates:								
Average		0.04	0.04	0.12	0.03	0.14	0.72	0.05
Maximum		0.08	0.09	0.24	0.05	0.24	2.41	0.06
Variation in samples:								
Average		0.17	0.22	0.29	0.09	0.37		0.23
Maximum		0.18	0.25	0.30	0.09	0.46		0.25

¹ *This Journal*, 1926, 9: 219.

TABLE 7.
Results on analysis of samples.

COLLABORATOR	SAMPLE NUM- BER	MOISTURE		NON-FAT SOLIDS		FAT		
		Official	Proposed	Official	Proposed	Official		Proposed
						By dif- ference	Direct	
J. T. Keister	A*	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
		13.44	13.22	1.78	1.76	84.78	85.41	85.02
		13.43	13.13	1.68	1.78	84.89	85.43	85.09
	B*	13.41						
		16.26	16.08	2.99	3.25	80.75	80.60	80.67
		16.26	16.22	2.97	3.19	80.77	80.68	80.59
	C*	16.28	16.34		3.08			80.58
		13.27	13.05	2.56	2.72	84.17	84.09	84.23
		13.26	12.89	2.78	2.68	83.96	84.18	84.43
	A1†	13.23						
		13.15	12.61	1.74	1.68	85.11	85.47	85.71
		13.21	12.67	1.77	1.78	85.02	85.48	85.55
	B1†	16.45	16.26	2.85	3.20	80.70	80.74	80.54
		16.50	16.24	2.73	3.49	80.77	80.83	80.27
		16.45						
	C1†	13.22	12.94	2.64	2.71	84.14	84.13	84.35
		13.16	13.00	2.66	2.52	84.18	84.10	84.48
		13.22						
	A1‡	13.16	12.81	1.69	1.77	85.15	84.47	85.42
		13.12	12.92		1.71		84.70	85.37
	B2‡	16.41	16.22	3.03	3.26	80.56	80.45	80.52
		16.43	16.32	2.97	3.12	80.60	80.46	80.56
		16.34						
	C2‡	13.14	12.88	2.70	3.01	84.16	84.17	84.11
		13.15	12.87	2.63	2.81	84.22	84.11	84.32
	B*	16.09	15.84	2.86	3.25	80.99	81.05	80.91
		16.15	15.79	2.90	3.29	81.04		80.92
		16.06						
	C*	13.15	12.83	2.77	3.00	84.04	84.13	84.17
		13.11	12.87	2.80	2.66	84.09	84.10	84.47
		13.06						
W. D. Richardson	1†	16.40	16.54	4.00	4.26	79.60	79.20	79.20
		16.50	16.64	4.10	4.16	79.40	79.40	79.20
C. A. Roach	1†	15.95	15.97	2.52	2.50	81.53		81.53
		15.93	15.90	2.50	2.52	81.57		81.58
H. R. Smith	1†	15.98	15.93	3.21	3.32	80.81		80.75
		15.97	15.90	3.18	3.33	80.85		80.77
		15.95		3.17		80.88		
	2†	15.95	15.93	3.13	3.29	80.92		80.78
		15.94	15.90	3.19	3.30	80.87		80.80
		15.93		3.17		80.90		
	3†	15.91	15.90	3.28	3.27	80.81		80.83
		15.85	15.85	3.25	3.30	80.90		80.85
	1¶	15.65	15.41	4.05	3.91	80.30		80.68
		15.68	15.51	4.05	3.95	80.27		80.54
C. B. Stone	2¶	15.62	15.32	4.01	4.01	80.37		80.67
		15.57	15.28	4.02	3.93	80.41		80.79

* Official-shaking modification.

† Official-mixing modification.

‡ Proposed mechanical stirrer.

¶ Official-shaking modification, then mixed.

COMMENTS ON ANALYSIS OF SAMPLE BY COLLABORATORS.

Haynes, Hickey, and Treuthardt.—Sample No. 1 was a composite of seven subdivisions of a butter which, on previous analysis, showed an average moisture content of 16.00 per cent, non-fat residue 3.04 per cent, fat by difference 80.96 per cent, and salt 2.34 per cent. Sample No. 2 was a composite of 6 subdivisions of a butter which on previous analysis showed an average moisture content of 13.76 per cent, non-fat residue 2.82 per cent, fat by difference 83.75 per cent, and salt 2.02 per cent. Each of these samples was thoroughly mixed by the mechanical stirrer, and samples were weighed out by each analyst at about the same time. Haynes did well with the official direct determination of fat. The results of the others, however, lead us to believe that this method is not so accurate as the indirect method. At best, it presents no advantage over the latter. The results by the proposed crucible method show a tendency to high non-fat residue, suggesting a retention of moisture or fat by the alundum. This is shown more clearly in the two determinations made by Treuthardt on sample No. 1, where large size samples were taken, the results found being 15.60 and 15.64 per cent. We prefer the official method with indirect determination for routine work, especially since we do no further work on samples showing 15 per cent or less of moisture. We weigh the samples into 50 or 100 cc. beakers, which we find come to constant weight after heating 3–4 hours and which permit a near transfer of residue to the crucible. To avoid the formation of a crust of salt and casein in the beaker, add to the dried residue a small portion of petroleum ether and rotate at once until the fat is practically all dissolved. Allow to stand a minute and then rotate and transfer the entire contents at once into the Gooch crucible. This manipulation will remove practically all the residue from the beaker; any small remainder is easily transferred by a rubber-tipped rod and petroleum ether.

Keister.—(See comments under "Preparation of Sample".)

Richardson.—We find no advantage in the proposed method of analysis over the official method.

Roach.—The proposed sand-Gooch extraction method is much slower than the official method. It must be watched very closely, otherwise water will collect on the condensing flask and run down into the crucible and dissolve some salt.

Smith.—Three subdivisions of the same lot of butter were prepared as follows: 6 pounds of tub butter from one tub was melted in warm water with stirring until the entire mass was of a thick creamy consistency. It was then thoroughly stirred and poured into nine E. Z. seal, pint jars in approximately equal portions. Jars Nos. 1, 2, and 3 were analyzed by the official method and also by the proposed alundum method. The alundum method worked well. The continuous extractor operated effectively. Rather than have a flow of water through the condensing flask, it was found that when once full of tap water the flask was sufficient cooling medium for the half-hour extraction period necessary for a single determination. It was also found that the prepared crucibles changed weight very little from one set of samples to the next. Therefore, a series of crucibles was prepared by adding the necessary amount of alundum to bring the final weight of each to a convenient even figure, viz., 30.010 grams. This facilitated rapid weighing.

Stone.—The sample was softened with the lid on tight at a temperature varying from 30° to 36°C. When partly melted it was shaken by hand to thoroughly mix the sample. The lid was then removed, and the sample was stirred with a spoon before being weighed. In the official method a 2 gram sample was used, while in the proposed method a 1 gram sample from the same jar was used. The moisture determination in the official method was heated for 3 hours at the temperature of boiling water. In Sample No. 1, 0.21 per cent more moisture was found by the official method than by the proposed method. Sample No. 2 gave 0.30 per cent more moisture by the official method than by the pro-

posed method. Judging from these results it might be possible that 2 hours is not sufficient for moisture determinations in the proposed method. Of course two determinations are not sufficient to pass judgment on any analytical procedure. Owing to the fact that both methods determine the fat indirectly, the proposed method would give a higher result. It is rather difficult in the proposed method to weigh up the sample in the Gooch crucible, and since the crucible is three-fourths full of the granular alundum, it is also difficult to remove butter from the crucible should an excess be placed therein. There is a possibility of removing some of the alundum when the butter is being removed.

DISCUSSION.

In general, the variation in check results by the proposed method is considerably less than that by the official method, particularly in the non-fat solids. With one exception, the collaborators are agreed that the proposed method is more rapid and more convenient than the official method.

The fact that some of the collaborators obtained lower moisture results and higher non-fat solids results by the proposed method than by the official method indicated that the drying time of 2 hours, under their laboratory conditions, was not sufficient, although this had not been found to be the case in the associate referee's laboratory where a uniform temperature of 105°C. is used.

RECOMMENDATIONS FOR ANALYSIS.

It is recommended that the proposed method of analysis be made a tentative method and that it be further studied.

REPORT ON CHEESE.

By E. O. HUEBNER¹ (Wisconsin Dairy and Food Commission, Madison, Wis.), *Associate Referee*.

Certain chemicals, known to the trade as emulsifying agents, are used in the manufacture of process cheese to aid in producing a uniform texture and to prevent the final product from becoming grainy and gritty. Although a number of chemicals have been proposed for this purpose, only three are in common use at the present time. These are sodium potassium tartrate (Rochelle salt), sodium citrate, and disodium phosphate.

The quantities of these chemicals that may be added in the manufacture of this product are limited specifically by law in one state, and their use is further limited by the fact that large quantities would reduce the fat content of the cheese solids below the legal minimum limits which process cheese must meet in other channels of trade.

Since the practice of using emulsifying agents in making process cheese is general, methods for their detection and estimation are of considerable

¹ Presented by W. B. Griem.

importance to agencies charged with the enforcement of food laws. As a result of a recommendation adopted by the Association of Official Agricultural Chemists, that methods of analyses for these emulsifying agents be studied, methods for the detection and estimation of tartaric acid, citric acid, and added phosphates are presented. The Food Control Laboratory of the Food, Drug and Insecticide Administration, United States Department of Agriculture, has developed workable methods for the determination of tartaric and citric acid in process cheese. The procedures for these emulsifying agents used by the associate referee are those outlined by Keister and Hartmann of that laboratory.

TARTARIC ACID.

The method for tartaric acid is an adaptation of the official method for total tartaric acid to process cheese. The procedure has been published¹.

Results obtained by different analysts of the Food Control Laboratory on samples of cheeses processed in that laboratory were most satisfactory. In a private communication Keister presented figures which indicated a high percentage of recovery of tartaric acid as well as excellent agreement of results by three analysts. In working with the method in this laboratory, samples of cheese were prepared for analysis and then definite quantities of tartaric acid were added, so that the conditions of analysis simulated those of the analysis of process cheese. That the procedure permits of the recovery of the chemical to a remarkable degree will be noted from the following table, in which are given the percentages of added and of recovered tartaric acid.

TARTARIC ACID ADDED	TARTARIC ACID RECOVERED
<i>per cent</i>	<i>per cent</i>
0.59	0.56
	0.56
0.85	0.85
	0.85
1.08	1.03
	1.05
1.19	1.14
	1.18
1.51	1.44
	1.45
1.77	1.78
2.02	2.01
	2.02
2.21	2.11
	2.11
2.55	2.51
	2.53

These figures are in agreement with the results obtained in the Food

¹ *This Journal*, 1928, 11: 40.

Control Laboratory and indicate that within the range used in the manufacture of process cheese the recovery is very satisfactory. Since the molecular weight of sodium potassium tartrate is nearly twice that of tartaric acid it will be seen that the higher figures in the table represent a larger quantity of emulsifier than is ordinarily used.

CITRIC ACID.

The tentative A. O. A. C. method for citric acid in fruit products is the basis for the Keister-Hartmann method. Some of the modifications recommended by Hartmann and Hillig¹ were incorporated. This method has also been published².

Cheese samples were prepared for analysis, and then definite quantities of citric acid were added. The results, as shown in the table, indicate that a satisfactory degree of recovery can be obtained. The columns represent the percentages of anhydrous citric acid added and the percentages recovered.

CITRIC ACID ADDED	CITRIC ACID RECOVERED
<i>per cent</i>	<i>per cent</i>
0.49	0.53
	0.53
0.70	0.69
	0.66
0.80	0.77
	0.79
1.00	0.94
	0.95
1.47	1.33
	1.35

The relation of sodium citrate to citric acid is such that one part of anhydrous citric acid is equivalent to 1.86 parts of sodium citrate. On that basis 1.47 per cent of anhydrous citric acid is equivalent to 2.73 per cent of sodium citrate. Inspection has failed to reveal the use of that emulsifying agent to such an extent.

ADDED PHOSPHATES.

In a series of determinations on the quantities of calcium oxide and phosphorus pentoxide in various types of cheese, I. M. Williams³ of this Department found that the phosphorus pentoxide-calcium oxide ratio for American, Brick, and Swiss varied slightly, but that for each type the ratio was fairly constant and could be used as a basis for computing the quantity of added phosphates. The average amounts of calcium oxide and phosphorus pentoxide, together with the P_2O_5/CaO ratio for the different types of cheese, are summarized in the following table:

¹ *This Journal*, 1927, 10: 264.

² *Ibid.*, 1928 11: 41.

³ *Ibid.*, 1927, 10: 302.

		P ₂ O ₅ per cent	CaO per cent	P ₂ O ₅ /CaO ratio
American	(17 samples)	1.11	1.01	1.094
Brick	(12 samples)	1.20	1.09	1.096
Swiss	(11 samples)	1.35	1.29	1.043

It will be noted that the relation of phosphorus pentoxide and calcium oxide is practically the same in the case of Brick cheese as in the case of American cheese. In the case of Swiss cheese both values are higher, but the quantity of calcium oxide is proportionately higher than in the other two types. High values for phosphorus pentoxide with a normal calcium oxide content for a certain type of process cheese indicate the addition of phosphates. The increased pentoxide content may be calculated to disodium phosphate.

In view of the fact that in many cases process cheese is a blend of different types with one type predominating, it would be desirable to do further work on the phosphorus pentoxide and calcium oxide content of these blends.

RECOMMENDATIONS¹.

It is recommended—

(1) That the methods for tartaric and citric acids be the subject of collaborative study.

(2) That further work be done on the phosphorus pentoxide-calcium oxide ratio of process cheese.

No separate report on dried milk was given by the associate referee. The following joint report was presented:

JOINT REPORT ON MALTED MILK AND DRIED MILK.

By B. G. HARTMANN, *Associate Referee on Malted Milk*, and J. T. KEISTER, *Associate Referee on Dried Milk* (U. S. Food, Drug and Insecticide Administration, Washington, D. C.).

At the 1926 meeting the association recommended further study of methods for the determination of fat and moisture in malted milk and dried milk for the purpose of ascertaining whether these methods can be harmonized and used for the two products.

The last revision of *Methods of Analysis* does not include a section on the analysis of dried milk. It does, however, prescribe methods for the essential determinations in the examination of malted milk, and the methods for protein and ash have been adopted tentatively for dried milk².

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 76.

² *This Journal*, 1927, 10: 72.

Previous reports¹ of the associate referee have shown that when ammonia is used in the determination of fat in malted milk by the Roesse-Gottlieb procedure, consistently lower results are obtained than when the ammonia is omitted; whereas, in the case of dried milk, the results by the two procedures lead to no definite choice regarding the use or omission of ammonia. However, its use is recommended in dried milk because it gives a cleaner and less troublesome extraction.

Work this year on the determination of moisture shows that the water oven method, which at present is tentative for malted milk, is not suitable for either malted or dried milk; in fact, a comparative study of the water and vacuum oven methods showed that drying in vacuo yields a much higher percentage of moisture than drying in the water oven. The method used is essentially that adopted for cheese². The results of the study on moisture determinations are shown in the table.

The data show quite conclusively that drying in the water oven is not so effective as drying in vacuo. In reporting their results, the analysts called attention to the fact that drying in the water oven is invariably attended by a marked darkening of the sample, while in vacuo only a slight darkening is produced. In explanation of the somewhat discordant results presented in the table, it should be stated that several months intervened between the determinations made by the two analysts.

With respect to the recommendation for the study of the cold water extract of malted milk, the associate referee on this subject can see no advantage to be gained by conducting such an investigation for the sole purpose of judging a malted milk. Similarly, an investigation of the subject of carbohydrates seems unnecessary. The carbohydrates occurring in malted milk comprise a number of sugars—notably maltose, lactose, and dextrose, together with dextrans—all of which vary in their proportions with the process of manufacture. Just what would be gained by a knowledge of the actual percentage of these constituents is not apparent. The fat and moisture determinations, in conjunction with a microscopical identification of the sample, would seem to furnish the necessary information for judging a malted milk. The malted milks on the market that have been examined have been found true to standard. Since malted milk is being used as a basis for a number of products appearing extensively on the market, such as the sweetened chocolate flavored mixtures, it is realized that methods for determining added sugar in these products might be of advantage.

A microscopical method for differentiating between true malted milk and mechanical mixtures (dried milk and dried malt extract) has been formulated. The work is not ready for publication. It may be stated

¹ *This Journal*, 1923, 6: 435; 1924, 8: 14.

² *Ibid.*, 1925, 9: 44.

Results of moisture determinations in malted milk and milk powders obtained by the water oven and vacuum oven methods.

DESCRIPTION OF SAMPLE	J. T. KEISTER				J. I. PALMORE			
	Drying in Water Oven	Drying in Vacuum Oven			Drying in Water Oven	Drying in Vacuum Oven		
		3 Hours	5 Hours	6 Hours		3 Hours	5 Hours	6 Hours
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Malted Milk-A	2.66	2.90	3.09	3.04	2.56	2.92	3.24	2.88
	2.59	3.02	3.06	3.08	2.12	3.10	3.02	2.88
	2.56	3.03	3.03	3.05	2.29	3.23	3.02	2.91
Average	2.60	2.98	3.06	3.06	2.32	3.08	3.09	2.89
Malted Milk-B	2.41	2.82	3.00	3.12	2.51	3.26	3.26	3.24
	2.51	2.73	3.02	3.18	2.80	3.24	3.13	3.15
	2.52	2.93	3.14	3.00	2.60	3.32	3.21	3.19
Average	2.48	2.83	3.05	3.10	2.64	3.27	3.20	3.19
Malted Milk-C	3.07	2.80	3.31	3.36	3.22	3.39	3.15	3.18
	3.21	2.83	3.32	3.31	3.06	3.22	3.22	3.23
	3.12	2.61	3.34	3.24	3.07	3.01	3.23	3.27
Average	3.13	2.75	3.32	3.30	3.12	3.21	3.20	3.23
Whole Milk Powder-A	0.97	1.59	1.78		0.69	1.54	1.54	1.67
	0.91	1.62	1.77		0.78	1.77	1.54	1.72
	0.84	1.66	1.63		0.68	1.66	1.62	1.70
Average	0.91	1.62	1.73		0.72	1.66	1.57	1.70
Whole Milk Powder-B	2.66	3.55	3.55		2.22	3.83	3.51	3.56
	2.72	3.51	3.49		2.36	3.60	3.61	3.64
	2.54	3.49	3.48		2.51	3.50	3.54	3.63
Average	2.64	3.52	3.51		2.36	3.64	3.55	3.28
Part Skim Milk Powder	2.41	2.79	3.06	2.87	1.67	2.89	2.79	2.99
	2.56	2.78	2.92	2.87	1.57	2.96	2.85	3.04
	2.79	3.01	2.96		1.66	2.86	2.83	2.88
Average	2.59	2.86	2.98	2.87	1.63	2.90	2.82	2.97
Skim Milk Powder	5.59	6.94	7.12		5.97	6.59	6.80	6.69
	5.63	6.95	7.14		6.05	6.54	6.78	6.65
		6.91	7.01		5.98	6.86	6.64	6.81
Average	5.61	6.93	7.09		6.00	6.66	6.74	6.72

at this time, however, that the microscope will definitely differentiate between true malted milks and assembled products.

CONCLUSION AND RECOMMENDATIONS¹.

The determination of moisture by the water oven method is not serviceable for malted milk and the procedure should be dropped from *Methods of Analysis*. It is recommended that the procedure adopted for

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 76.

cheese at the 1925 meeting be substituted, 1-1½ grams of material being used instead of 2-3 grams and the sample being dried 5 hours instead of 4 hours. This method is applicable also to dried milk, and its adoption is recommended.

The Roesse-Gottlieb procedure for the determination of fat cannot be unconditionally harmonized for malted milk and dried milk. The use of ammonia is preferred for dried milk, while for malted milk its omission is essential for maximum fat extraction. It is recommended that the procedure described previously¹, and which is now tentative for dried milk, be adopted as tentative for malted milk, with the provision that the ammonia be omitted.

While a study of the water-soluble extract and carbohydrates of malted milk is unnecessary for judging compliance with the established standard, in view of the fact that mixtures composed of malted milk, sugar, and a flavoring material are found in the market, a study of methods for determining the various sugars in such mixtures is desirable.

Since the identification of malted milk by the microscope has been established, it is recommended that further work be conducted tending toward its adoption at the next meeting.

REPORT ON ICE CREAM².

By L. H. McROBERTS³ (Food and Drug Laboratory, Bismarck, N. Dak.), *Associate Referee*.

Following the report of October, 1926⁴, relative to proposed methods for gelatin and ash in ice cream, the method for ash was submitted to fourteen collaborators. To date five reports have been received, the results of which are given in Table 1.

The modified Ferris method for gelatin outlined in the last report was used in this laboratory during the previous summer with some minor changes. Although not as yet in a form satisfactory for routine inspection analysis, it would be well to submit the method to trial. The chief objections are certain time-consuming operations and a possible error in transferring the final precipitate of gelatin unless great care is taken.

In connection with the method for ash, some thought was given to calculation of milk solids not fat on the basis of the average ratio of M. S. N. F./ash of normal milk and on the basis of the ratio of M. S. N. F./protein of normal milk.

Total counts of bacteria on all the samples of ice cream analyzed during

¹ *This Journal*, 1925, 8: 482.

² Presented by E. M. Bailey.

³ The writer wishes to acknowledge the collaboration of Roe E. Remington of the North Dakota Agricultural College, Fargo, N. Dak., who has been associated with this department for the purpose of conducting these investigations.

⁴ *This Journal*, 1927, 10: 315.

the previous summer were determined according to the method outlined in this report. On the basis of forty samples purchased from as many retail locations, counts were obtained ranging from 5,000 to 800,000 per cubic centimeter of the melted ice cream and an average value of 200,000 per cubic centimeter. No mention is made in the literature of a definite method for total count of bacteria in ice cream, and it seems that such a method should either be included in the methods of the A. O. A. C.¹ or in Standard Methods of Milk Analysis of the American Public Health Association.

DETERMINATION OF ASH IN ICE CREAM.

(Method submitted to collaborators and discussed in previous report.)

Use the residue from the fat determination according to the official Roese-Gottlieb method² as a basis for ash. To facilitate removal of the residue, use Pyrex tubes of 25 x 250 mm. for the fat determination instead of Röhrig tubes. These tubes are equipped as individual wash bottles (devices for blowing off the ether similar to those employed in the Werner-Schmidt method³).

After the last portion of the ether has been removed, place the tubes in a water bath and heat slowly until the ether and most of the alcohol have been expelled. (Frothing is kept down by a jet of air directed into the tubes.) Pour into a weighed dish, washing the tubes with small quantities of water, and evaporate to dryness on a water bath. (Particles adhering to the tubes can best be removed by a rubber-tipped glass rod.) Ignite at 500°C. and weigh.

From a comparison of the following methods as the best means of ashing ice cream: (1) Official method for milk and cream⁴; (2) official method for sweetened condensed milk⁵; and (3) the proposed method as outlined above, it was noted that in the majority of determinations the highest results were obtained by the proposed method. An increase of from 0.02 to 0.05 per cent could not be accounted for by blank determinations when the same apparatus and reagents were used. This difference would seem to be negligible were it not for the fact that it is desired to calculate milk solids not fat on the basis of the average ratio of M. S. N. F./ash of normal milk. According to tables published by Mojonner and Troy⁶, ash times 12.36 should give the milk solids not fat. A difference in 0.05 per cent ash would therefore result in a difference of about 0.6 per cent in milk solids not fat. According to the results of the collaborators compiled in Table 1, the majority of determinations for milk, cream, or sweetened condensed milk by the proposed method gave higher results than by the official methods.

According to results obtained by the official methods, columns (A) and (B), the addition of nitric acid does not make a difference. In the experience of the associate referee, the same care is required to avoid

¹ *Methods of Analysis*, A. O. A. C., 1925.

² *Ibid.*, 280.

³ Leach, *Food Inspection and Analysis*, 1920, p. 126.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 206.

⁵ *Ibid.*, 274.

⁶ *The Technical Control of Dairy Products*, p. 15.

spattering with or without the acid, and considering the precaution necessary in fuming off the excess, its use might well be eliminated from the procedure.

COMMENTS BY COLLABORATORS.

A. S. Wells.—The samples of ice cream used represent a fair average of those sold on the markets of the State of Oregon. I find that the results of close checking on the three different methods depend, as stated in your letter of instruction, on absolute control of heat in igniting the residues. Samples 6 and 7 were carefully controlled and results were the most satisfactory.

After experimenting on the seven samples of ice cream as tabulated, I consider the proposed ashing method superior to the methods now in use and think it will be eventually adopted as official.

E. O. Huebner.—After drying and preliminary charring, the final ashing was in all cases made in a muffle at a temperature at or below 500°C. A standardized thermocouple, accurate to within 10°C., was used. I had no trouble in obtaining an ash free from carbonaceous matter.

For removing the fat according to the proposed method, I used a Majonnier extraction tube made of Pyrex glass. Results by this method are uniformly higher than those by the official method.

W. B. White.—* * * We did not use nitric acid in the direct method. The A. O. A. C. methods are inconsistent on this point, calling for nitric acid in milk and cream, but none in concentrated milk products. Personally I am against varying the ashing process by introducing any reagents whatever if good results can be obtained without them. We are of the opinion here that good results can be obtained on dairy products, whether sweetened or not, if the fat is carefully burned off before the dish is placed in the muffle. It would seem that the careful analyst would always take such a precaution to avoid a sudden explosive ignition which often occurs in the muffle when such a precaution is not observed.

The blank on the Mojonnier reagents was nil.

It is our opinion that the proposed method will give just as accurate results as direct ignition, but it is tedious and troublesome on account of the gelatinous ring which forms in the flask during evaporation. It took considerable shaking and scraping with a copper wire to loosen the ring.

J. W. Kellogg.—The results obtained show the proposed method to give slightly higher results.

The difference in the results between the proposed method and the official A. O. A. C. method, when nitric acid is used as directed, is so slight that any distinct advantage of the proposed method over the official method is not apparent.

In carrying out the official test there was no loss due to effervescence when evaporating to dryness after the addition of nitric acid; this was avoided by using deep platinum dishes.

The proposed method has possibilities for error not found in the official method, i. e. in transferring the weighed ice cream mixture to the Roesse-Gottlieb tube, and after extraction of fat, in transferring the mixture to the platinum dish. It requires much more time and it is also more expensive owing to the chemicals used in the fat extraction. The very slight increase in ash obtained is not sufficient to warrant its adoption in place of the very simple official method.

Considering the fact that only slightly higher results were obtained by the proposed method, the adverse comments of the collaborators are justified on the basis of ash as a unit determination. If, however, the

TABLE 1.
Collaborative results on ash in ice cream.

COLLABORATOR	(A)	(B)	(C)	(D)	(E)	(F)		(G)
	OFFICIAL METHOD WHITE HNO ₃	DIRECT METHOD ASHING WITHOUT HNO ₃	PROPOSED METHOD RESIDUE OF FAT DETER- MINATION	FAT	TOTAL SOLIDS	(C) - (A)	(B) - (A)	
A. S. Wells Portland, Ore.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	Commercial ice cream
	0.62	0.57	0.60	14.0	38.2	0.02 -	0.05 -	" "
	1.14	1.11	1.10	14.9	39.6	0.04 -	0.03 -	" "
	0.84	0.78	0.72	11.4	36.2	0.12 -	0.06 -	" "
	0.85	0.81	0.71	12.0	36.6	0.14 -	0.04 -	" "
	1.02	1.00	0.96	9.5	39.1	0.06 -	0.02 -	" "
J. T. Keister U. S. Department of Agricul- ture, Washington, D. C.	0.91	0.87	1.05	12.5	38.3	0.14 +	0.04 -	" "
	0.58	0.59	0.60	10.8	0.02 +	0.01 +	Ice cream mix
	0.57	0.58	0.60	10.2	0.03 +	0.01 +	Ice cream mix
	0.46	0.48	0.50	22.3	0.02 +	0.00	" "
	0.67	0.69	0.72	13.7	0.05 +	0.02 +	" "
	0.71	0.71	0.73	11.8	0.02 +	0.00	Commercial ice cream
E. O. Huebner Dairy and Food Commission Madison, Wis.	0.76	0.71	0.75	9.8	0.01 -	0.05 -	" "
	0.90	0.92	0.92	0.02 +	0.02 +	Commercial ice cream
	1.05	1.06	1.07	0.02 +	0.01 +	" "
	0.95	0.97	0.96	0.01 +	0.02 +	" "
	0.92	0.93	0.94	0.02 +	0.01 +	" "
	0.48	0.50	0.51	0.03 +	0.02 +	Ice cream mix
W. B. White Department of Agriculture and Markets, Albany, N. Y.	0.55	0.56			Ice cream mix
	...		0.56			" "
J. W. Kellogg Bureau of Foods and Chem- istry, Harrisburg, Pa.	0.55	0.53	0.56	0.01 +	0.02 -	Ice cream mix

fact that a fat determination would doubtless be made is considered, there is the distinct advantage of one sample for both determinations. A correct ash determination is of most importance in the calculation of milk solids not fat, and as stated previously, a slight variation in the ash will make a large variation in milk solids not fat as calculated. It has not been shown that the increase in ash obtained by the proposed method is not justified.

MILK SOLIDS NOT FAT.

If a suitable method can be formulated for the determination of sucrose in ice cream, one of the means of determining milk solids not fat will be by difference [Total Solids - (Fat + Sucrose)]. This figure will be of value provided that such additions as fruit or nuts are removed before starting the analysis, a correction for gelatin is made, and the analyst is assured that the ice cream does not contain dextrose or other reducing sugars. A method of this sort would not be applicable to chocolate ice cream.

Considering the many interfering factors in a determination of milk solids not fat by difference, it would seem that for the present a method of calculation by ratio is the only solution of the difficulty.

Table 2 was arranged to show the possibilities of dependable ratios between the constituents of normal milk.

TABLE 2.
Average composition of normal milk.

COMPILED BY	M. S. N. F.	ASH	FAT	PROTEIN	CASEIN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1. Mojonniere and Troy	8.65	0.70	3.70	3.25	2.60
2. Babcock	9.10	0.70	3.60	3.80	3.00
3. Richmond	8.91	0.75	3.74	3.00
4. Van Slyke	9.00	0.70	3.90	2.50
5. Lythgoe	8.77	0.76	4.03	3.30
Average	8.89	0.72	3.80	3.45	2.78

1. The Technical Control of Dairy Products, p. 15. The data represent the composition of the mixed herd milk from 1217 different herds. They include the different breeds and classes of cows in central and western New York. The number of cows in the different herds is not known but would probably average about ten.

2. N. Y. State Agr. Exp. Sta., Tech. Bull. 39, 1914.

3. Richmond's Dairy Chemistry. Gives average results showing the composition of milk in England, calculated from about 280,000 analyses, covering a period of 17 years.

4. Modern Methods of Testing Milk and Milk Products. Gives the average of results on over 5,000 samples of American milks.

5. *J. Ind. Eng. Chem.*, 1914, 6: 899. The Composition of Milk as Shown by Analyses of Samples of Known Purity Made by The Massachusetts State Board of Health. Analyses of 600-700 samples, 434 of which came from individual cows and the balance from herds.

In the experience of R. E. Remington and the associate referee in connection with this work, it would seem that the values of 0.75 and 0.76

per cent for ash are outside the normal range. Methods of ashing may have been responsible.

The following ratios have been calculated from the average of the results of the five authorities listed above.

M. S. N. F./ash	-	12.35
M. S. N. F./protein	-	2.58
Protein/ash	-	4.79

The M. S. N. F./ash ratio presents the least difficulty because no corrections are needed and the ash figure is doubtless the most constant. Whether or not the ash figure could be depended upon in case neutralizers were used would be determined by calculating the protein/ash ratio. If lime-water or milk of magnesia is used as an anti-acid, this ratio will have an abnormally low value.

TOTAL NITROGEN.

The Kjeldahl-Gunning-Arnold Method¹ was found to be most satisfactory for ice cream.

Weigh 4-5 grams of the sample prepared as directed² and transfer to a Kjeldahl flask. (The sample weight is best obtained and the transfer made by the use of a small weighing flask equipped with glass and rubber tube connection for drawing off the required portion.) Correct the percentage of nitrogen for the nitrogen obtained from gelatin or other foreign proteins and multiply the result by 6.38 to obtain the percentage of milk proteins.

SUGARS.

No work has been done by the associate referee on the estimation of sugars. There is need for lactose-sucrose tables based on lactose-sucrose ratios that approximate the composition of these two ingredients of ice cream; that is, 1 part lactose to 2.5 parts sucrose. As noted previously, a determination of sucrose in ice cream would aid in the calculation of milk solids not fat and any revision of the present sugar tables should therefore take the lactose-sucrose ratio in ice cream into account. Other reducing substances, such as lactic acid, aldehydes, etc., must also be considered.

BACTERIOLOGICAL EXAMINATION OF ICE CREAM.

The following method is outlined for comment and decision by the committee as to whether a method of this nature should be included in the methods of the A. O. A. C. or referred to the proper authority. This method is now in use in the laboratory of the associate referee.

BACTERIOLOGICAL EXAMINATION OF ICE CREAM³.

OBTAINING THE SAMPLES.

Obtain samples as nearly as possible under the conditions in which they are sold to the consumer at retail. Take samples from drug stores, confectionery stores, cafes,

¹ *Methods of Analysis*, A. O. A. C., 1925, 8.

² *Ibid.*, 279, par. 88.

³ E. M. Stanton, bacteriologist, Office of State Food Commissioner and Chemist, Bismarck, N. D.

lunch rooms, ice cream parlors, etc., using for the purpose the ice cream dipper or scoop that is in daily use at the place of sampling. Take one sample from the center and one from each side. Place the three samples in a sterile half-pint glass fruit jar having a glass cover which is fastened by a wire clamp on top. Label each jar and place in a portable ice chest for transportation to the laboratory. When ice cream is sold in half-pint, pint, and quart packages, obtain an unbroken package of the desired size and transport to the laboratory in an iced chest.

METHOD.

Melt the contents of the glass jars by placing in a pan of warm water at 40°C.; after the ice cream has melted, shake the jar to mix the contents and with a sterile 1 cc. pipet make the desired dilution and plate. In the case of sealed packages, open them carefully and with a sterile spoon remove one portion from the center and one from each side of the package. Place the spoon samples in a sterile beaker, melt, and make dilutions with a 1 cc. sterile pipet and plate.

MEDIA.

Use agar as specified by the American Public Health Association in Standard Methods of Milk Analysis. Incubate and count the plates the same as for milk.

RECOMMENDATIONS¹.

It is recommended—

(1) That the proposed method for ash in ice cream be submitted to further collaborative study in connection with its value as a basis for the calculation of milk solids not fat.

(2) That a collaborative study be made of the average composition of normal milk in an effort to produce all available data on the subject and thereby establish ratios as a basis for the calculation of milk solids not fat in ice cream.

(3) That the modified Ferris method² for gelatin in ice cream be submitted to collaborative study.

(4) That since the Kjeldahl-Gunning-Arnold method was found to be satisfactory for the determination of total nitrogen in ice cream, it be adopted as a tentative method in the analysis of ice cream.

REPORT ON MILK PROTEINS.

By H. C. WATERMAN (Office of Experiment Stations, Washington, D. C.), *Associate Referee*.

The Associate Referee for Milk Proteins attempted to secure collaborative study of a method for the determination of casein in fresh milk³, but was able to get results from two collaborators only, partly because of the shortness of the time remaining when it became possible to send out the request.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1927, 11: 77.

² *This Journal*, 1927, 10: 317.

³ *Ibid.*, 259.

J. T. Keister of the Food and Drug Administration secured satisfactory figures but found the filtration unsatisfactory. The filtrates from the casein precipitates were not clear. He proposes a slight change in the filtration which greatly lessens this trouble. W. B. White of the New York Department of Agriculture and Markets also reported trouble in getting clear filtrates, but he had no time to study the correction of this point. The associate referee recommends¹, therefore, the further study of the method with a change in the directions to include Keister's improvement in the filtration. Keister's report notes as an advantage of the proposed method that the washing of the casein precipitate is eliminated.

Results of casein determinations in milk obtained by Keister.
(Expressed as grams per 100 cc.)

SAMPLE	TOTAL PROTEIN "A" $N \times 6.38$	CASEIN OFFICIAL METHOD	CASEIN BY PROPOSED METHOD		DIFFERENCE AVERAGE
			PROTEIN IN FILTRATE "B"	CASEIN (A - B)	
A	3.21	2.54	0.84	2.39	-0.14
	3.24	2.55	0.82	2.41	
			0.82	2.41	
B	3.28	2.49	0.84	2.45	-0.04
	3.30	2.54	0.83	2.46	
			0.79	2.50	
C	3.24	2.50	0.83	2.39	-0.13
	3.20	2.54	0.84	2.38	

REPORT ON QUALITATIVE TESTS FOR DAIRY PRODUCTS.

Detection of Gelatin in Milk.

By SYDNEY H. HALL² (State Department of Public Health, Boston, Mass.), *Associate Referee.*

J. Hortvet, the former referee on dairy products, suggested making a study of methods for detecting in milk such added substances as gelatin, sucrose, glycerine, and chlorides. Of these, gelatin alone was studied.

The following paragraph regarding the official method for gelatin³ was taken from a communication received by the association from W. D. Bigelow, Director of the Research Laboratories, National Canners Association:

We found that this method gave a positive test for gelatin with all of the well-known brands of evaporated milk which we purchased on the Washington market. The precipitate obtained was of a flocculent nature that settled readily and which differed from

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 77.

² Presented by H. C. Lythgoe.

³ *Methods of Analysis*, A. O. A. C., 1925, 269.

that obtained with the same milk to which 1/10 per cent gelatin had been added. It was further found, however, that a negative test for gelatin was obtained when the evaporated milk was diluted one to one with water, in order to make it comparable in concentration with fresh milk. However, a slight flocculent precipitate settled out on long standing (2-3 hours); a similar precipitate was obtained with fresh milk that had been heated either at the temperature of boiling water or at 140°F. Fresh milk itself gave a similar precipitate on standing overnight. From our recent experience it would seem advisable to insert in the method a paragraph on the preparation of the solution upon which the test is to be made. Of course, one would scarcely look for gelatin as an adulterant of sterilized evaporated milk, since gelatin is hydrolyzed at the temperature of boiling water and its thickening power destroyed. However, in the present case we may be somewhat embarrassed by the method as it is now given, as a foreign country is objecting to the importation of a shipment of American evaporated milk on the ground that it contains added gelatin.

Results as described by Bigelow were obtained from fresh, heated, and evaporated milk. A chemist well acquainted with the gelatin picrate precipitate would not, however, mistake this very flocculent precipitate which forms on long standing as a positive test for gelatin. Gelatin picrate comes down quickly and resembles barium sulfate in appearance except for its yellow color. In the case of a sour milk, however, the precipitate appears at once and is sufficiently fine to allow a chance for error.

M. Berrár¹ states that one part of concentrated aqueous solution of picric acid and four parts of alcohol will precipitate proteins, albumose, peptones, mucin, and casein, but will not precipitate gelatin. Then the gelatin may be precipitated from the filtrate by an excess of picric acid. When this procedure was applied to milk, a precipitate resulted when the excess of picric acid was added, even in the absence of gelatin.

Seidenberg² proposed a modification of the official method as follows: To the acid mercurous nitrate filtrate obtained as in the official method add an equal volume of saturated picric acid solution and shake very thoroughly to coalesce the gelatin picrate. Filter, wash the precipitate with very dilute ammonia (2-3 drops of concentrated ammonium hydroxide per 100 cc.) until the washings are slightly alkaline to litmus, and then with distilled water until the washings are neutral to litmus. This removes all excess of picric acid. Boil the filter paper and the precipitate in 10-20 cc. of distilled water. Filter hot, cool the filtrate, and add an equal volume of saturated picric acid solution. If gelatin was present in the original sample, a fine precipitate of the texture of barium sulfate precipitate forms immediately or within a few minutes. The misleading precipitate which appears in the first picric acid precipitation with a sour sample is absent in the second precipitation, separation being effected by its insolubility in hot neutral water. The gelatin picrate is soluble in hot neutral water.

It is recommended that the method be assigned for collaborative work³.

¹ *Biochem. Z.*, 1912, 47: 189.

² *J. Ind. Eng. Chem.*, 1913, 5: 927.

³ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 78.

REPORT ON FATS AND OILS.

By G. S. JAMIESON (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*.

During the past year the Thomas-Yu¹ method for the approximate determination of peanut oil in admixture with olive oil was further studied, together with Evers' modification² of the Bellier method³. The separation and determination of saturated and unsaturated fatty acids by the lead-salt-ether method⁴ and the determination of saturated fatty acids by the lead-salt-alcohol procedure of Twitchell⁵ were also studied.

As the Thomas-Yu method was described in last year's report⁶, it is not necessary to repeat it. The modified Bellier method for the detection and estimation of peanut oil in olive oil is as follows:

DETECTION AND ESTIMATION OF PEANUT OIL IN OLIVE OIL.

Modified Bellier Method.

REAGENTS.

Alcoholic potash.—To purify the alcohol add 1.5 grams of silver nitrate, dissolved in 3 cc. of water, to 1 liter of 95 per cent alcohol. Dissolve 3.0 grams of potassium hydroxide in 15 cc. of hot 95 per cent alcohol, cool, and add to the first solution. Shake and allow to stand until the precipitate has completely settled (at least overnight). Decant or filter the alcohol for use. Dissolve 80 grams of potassium hydroxide in 80 cc. of water and dilute to 1 liter with the purified alcohol.

Dilute acetic acid.—Add one volume of glacial acetic acid to two volumes of water.

Alcohol, 70 per cent by volume, acidified with hydrochloric acid.—To 700 cc. of 95 per cent alcohol add 10 cc. of concentrated hydrochloric acid and dilute to 950 cc. with water.

Alcohol, 90 per cent by volume.—Dilute 900 cc. of 95 per cent alcohol to 950 cc. with water. (Denatured alcohol, formula 30, may be used instead of ordinary 95 per cent alcohol for making up reagents.)

PROCEDURE.

Weigh out 5 grams of the oil into a saponification flask, add 25 cc. of the alcoholic potash, and saponify for about 5 minutes under a reflux condenser. Acidify the hot soap solution with 7.5 cc. of the diluted acetic acid and add 100 cc. of the 70 per cent alcohol reagent. Cool to 12°–14°C. for 1 hour. Filter and wash with the 70 per cent alcohol reagent at 17°–19°C., breaking up the precipitate occasionally by means of a platinum wire bent into a loop. Continue the washing until the filtrate gives no turbidity with water, and measure the washings. Dissolve the precipitate, according to its bulk, in 25–70 cc. of hot 90 per cent alcohol, and cool to a fixed temperature between 15° and 20°C. If crystals appear in any quantity, allow to stand at this temperature for 1–3 hours, filter, wash with a measured volume of 90 per cent alcohol (about half the volume used for crystallization), and finally with 50 cc. of the 70 per cent alcohol reagent. Wash the crystals with warm ether into a weighed flask, distil off the ether,

¹ *J. Am. Chem. Soc.*, 1923, 45: 113.

² *Analyst*, 1912, 37: 487.

³ *Ann. Chim. anal.*, 1899, 4: 4.

⁴ *Cotton Oil Press*, 1922, 6, No. 1, 41.

⁵ *J. Ind. Eng. Chem.*, 1921, 13: 806.

⁶ *This Journal*, 1927, 10: 323.

dry at 100°C., and weigh. If the melting-point is lower than 71°C., recrystallize from 90 per cent alcohol. Add the correction for the solubility in 90 per cent alcohol from Table A and also for the total volume of 70 per cent alcohol used in precipitating and washing (including the 100 cc. added in the first instance) from Table B.

If no crystals form from 90 per cent alcohol, or if a small quantity only is present, add a sufficient quantity of water to reduce the strength of the alcohol to 70 per cent (31 cc. water to 100 cc. 90 per cent alcohol). Crystallize at 17°–19°C. for 1 hour, filter, wash with the 70 per cent alcohol reagent, and weigh as before, adding the correction for the 70 per cent alcohol from Table B. If the melting point is below 71°C., recrystallize from a small quantity of 90 per cent alcohol, or again from 70 per cent alcohol.

The factor for converting the percentage of fatty acids to peanut oil varies with the melting point of the fatty acids and is given in Table B.

TABLE A*.
Correction factors — 90% alcohol.

WEIGHT OF MIXED ACIDS OBTAINED	CORRECTION TO BE ADDED PER 100 CC. OF 90% ALCOHOL USED FOR CRYSTALLIZATION AND WASHING AT—		
	15°C.	17.5°C.	20°C.
<i>grams</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>
0.05	+0.031	+0.040	+0.046
0.10	0.036	0.045	0.052
0.20	0.048	0.056	0.062
0.30	0.055	0.064	0.071
0.40	0.061	0.071	0.078
0.50	0.064	0.076	0.084
0.60	0.066	0.080	0.088
0.70	0.067	0.082	0.090
0.80	0.069	0.083	0.092
0.90	0.070	0.084	0.092
1.00	0.071	0.084	0.091
2.70	0.073	0.082	0.091

* Allen. Commercial Organic Analysis, 4th ed., vol. 2, p. 96.

TABLE B.
Correction factors — 70% alcohol.

WEIGHT OF ACIDS (CORRECTED FOR 90 PER CENT ALCOHOL)	CORRECTION PER 100 CC., 70 PER CENT ALCOHOL		
	MELTING POINT		
	71°C.	72°C.	73°C.
<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>
Above 0.10	0.013	0.008	0.006
0.08–0.10	0.011	0.007	0.006
0.05–0.08	0.009	0.007	0.005
0.02–0.05	0.007	0.006	0.005
Less than 0.02	0.006	0.005	0.004
Factor for conversion of percentage of fatty acids to peanut oil	17	20	22

The results obtained by the Thomas-Yu method and by the modified Bellier methods are given in Table 1.

TABLE 1.

Collaborative results.

(Percentage of peanut oil found.)

ANALYST	THOMAS-YU METHOD			EVERS MODIFICATION OF THE BELLIER METHOD		
	Oil No. 1*	Oil No. 2†	Oil No. 3‡	Oil No. 1*	Oil No. 2†	Oil No. 3‡
W. D. Richardson	31.46	18.80	none	31.75	19.72	none
	31.38	18.48		32.18	21.05	
M. L. Sheely	34.11	24.60	none	36.38	31.39	none
	34.52	23.94		36.72	31.40	

* 25% peanut oil.

† 15% peanut oil.

‡ 100% olive oil.

It will be observed that the results obtained by both methods for samples 1 and 2 are high. Last year six collaborators also reported high results, particularly in the sample which contained 25 per cent of peanut oil. It is evident that neither of these methods as now formulated will give satisfactory results in mixtures that contain 15 per cent or more of peanut oil. Therefore, it is recommended that no further study be made of these methods during the coming year.

DETERMINATION OF SATURATED AND UNSATURATED FATTY ACIDS.

Three samples (No. 1—corn oil, No. 2—cottonseed oil, and No. 3—peanut oil) were sent to the collaborators for analysis by the lead-salt-ether and the lead-salt-alcohol methods, which were as follows:

Lead-Salt-Ether Method.

Weigh such a quantity of oil as contains about 2 grams of saturated acids, in no case using more than a 20 gram sample, into a 200 cc. Erlenmeyer flask, and add 30 cc. of 95 per cent alcohol and a 25 per cent excess of a I-I aqueous solution of potassium hydroxide. Heat on the steam bath until saponification is complete (about one-half hour). Add a slight excess of dilute acetic acid, using phenolphthalein as an indicator, and bring back to a faint pink color with a dilute potassium hydroxide solution. Measure a quantity of 20 per cent lead acetate solution (60 cc. if a 10 gram sample is used, 120 cc. for a 20 gram sample) into a liter Erlenmeyer flask, add the same quantity of water, heat to boiling, and pour the neutralized soap solution carefully into the boiling lead acetate solution. Rinse the saponification flask with 3-4 cc. of alcohol, then with water, adding the washings to the hot solution. Continue the boiling for about 5 minutes, shake thoroughly, and cool under the tap, rotating the flask continuously so as to cause the lead soap to adhere to the sides and bottom of the flask. Pour off the cold aqueous solution, taking care to lose none of the lead soap. (Usually the solution is only slightly cloudy with basic lead acetate and no particles of lead soap are to be seen.) Wash two or three times with cold tap water, drain, and remove the last drops of water by means of a thin roll of filter paper or a wad of cotton held by the forceps. Do not expose the lead salts to the air any longer than necessary because they slowly absorb oxygen. Add a quantity of ether (100 cc. if a 10 gram sample is used, 200 cc. for a 20 gram sample) to the flask, shake, and warm cautiously until the lead soap is entirely loosened from the flask and completely disintegrated. Rinse down the sides

of the flask with a small quantity of ether, cork loosely, and place in the ice box overnight. Place a 7 cm. ordinary filter paper in a 7.5 cm. diameter Büchner funnel. Cut down a 9 cm. hardened paper so that its diameter is 4 or 5 mm. wider than the diameter of the funnel. Fit this hardened paper after wetting snugly into the funnel on top of the other paper, and dry with alcohol and ether. Decant the lead-soap mixture through this filter, using just enough suction to draw the liquid through. (Too much suction causes the moisture in the mixture to freeze and clog the filter, because the rapid evaporation of the ether lowers the temperature.) Transfer as much of the precipitate as possible to the filter, rinse out the flask, and wash the precipitate 6-8 times with ether, stirring with a small horn spoon. (Care should be taken to keep the lead soap slightly moist; otherwise it is difficult to remove it from the filter paper.) Transfer as much of the lead soap as possible to a 500 cc. separatory funnel with a spatula and wash in the adhering bits with a stream of ether. Drop the filter paper into the flask. Shake the contents of the separator until the lumps of lead soap are disintegrated. Then add about 20 cc. of concentrated hydrochloric acid and shake until the soap is decomposed. Add a few cc. of hydrochloric acid and water to the flask in order to decompose the soap that continues to adhere to the flask and filter paper; then wash into the separator with alternate portions of ether and water until no more particles of lead chloride or soap remain. Add 50 cc. of water, shake the contents of the separator, and after settling draw off the aqueous layer, being careful not to lose any particles of undecomposed lead soap. If the soap is not all decomposed, shake again with 10 cc. of acid. Wash the ether solution with successive 75 cc. portions of distilled water until the washings remain clear when the silver nitrate solution is added. Dehydrate the ether solution with 6-7 grams of anhydrous sodium sulfate. Then pour as much as possible of the solution from the neck of the separator into a weighed 300 cc. Erlenmeyer flask and run the remainder from the stem through a small filter into the flask. Wash the separator and sodium sulfate with several portions of ether and run through the small filter into the flask. Distil off the ether, heat in an oven at about 110°C. until the weight is constant, and weigh the saturated acid fraction thus obtained.

Transfer the ether solution of the soluble lead soap to a 500 cc. separator, shake with 20 cc. of concentrated hydrochloric acid until the lead soap is decomposed, add 75 cc. of water, and again shake. After settling, run an aqueous layer into a beaker. (Sometimes drops of the ether solution are trapped in the lead chloride precipitate and run off with the aqueous layer.) If drops of fatty acid float on the surface of the solution in the beaker, add a few cubic centimeters of ether and pour back into the separator, being careful to retain the bulk of the lead chloride precipitate in the beaker. Wash the ether solution with successive 75 cc. portions of distilled water until the washings remain clear when a silver nitrate solution is added.

Dehydrate with about 2 grams of anhydrous sodium sulfate and transfer to a 300 cc. weighed Erlenmeyer flask according to the directions for the saturated acid determination. Distil off most of the ether and remove the remainder by heating in an oven at 115°-120°C. for at least an hour while passing a stream of carbon dioxide through the flask. Cool in an atmosphere of carbon dioxide. Then remove the carbon dioxide from the flask by placing it under a bell jar connected with a vacuum system and exhausting the jar a number of times. Weigh the unsaturated acid fraction thus obtained.

Determine the iodine numbers of both saturated acid and unsaturated acid fractions. The iodine number of the saturated acid fraction, usually between 5 and 10, is a measure of the amount of unsaturated acids that contaminates the saturated acid fraction. Calculate the correction for the contaminating unsaturated acids as follows:

$$\frac{\text{Iodine No. of saturated acids} \times 100}{\text{Iodine No. of unsaturated acids}} = A \quad \left(\begin{array}{l} \text{percentage of unsaturated acids con-} \\ \text{taminating the saturated acid fraction.} \end{array} \right)$$

The proper correction is then obtained by means of the formula $\frac{A \times B}{100}$, in which B is the percentage of impure saturated acids. This correction is subtracted from the percentage of impure saturated acids and added to the percentage of unsaturated acids actually determined.

This method for the separation of the saturated and unsaturated fatty acids is not applicable to the analysis of fats and oils that contain erucic, chaulmoogric, hydnocarpic, or similar acids, because the lead salts of these acids are difficultly soluble in ether and this also applies to hydrogenated products that contain notable quantities of iso-oleic acid. Also the method is not adapted to the analysis of coconut or palm kernel oils that contain notable quantities of the lower fatty acids which give ether-soluble lead salts.

DETERMINATION OF SATURATED FATTY ACIDS WITH LEAD ACETATE IN ALCOHOLIC SOLUTION.

Separate the fatty acids from approximately 50 grams of the oil in the usual manner. Weigh in a beaker as much of the fatty acid mixture as is estimated to contain 1-1.5 grams of solid acids. (In case of a very liquid oil this quantity will be about 10 grams, while in the case of a tallow it will be only 2-3 grams.) Dissolve in 95 per cent alcohol.

Dissolve 1.5 grams of lead acetate in 95 per cent alcohol (Alcohol denatured with methyl alcohol may be used in place of 95 per cent alcohol. The total alcohol for the two solutions should be about 100 cc.) Heat both solutions to boiling and pour the lead acetate solution into the solution of fatty acids. Allow to cool slowly to room temperature and then allow to remain in the ice box for several hours, preferably overnight. Filter through a plain filter and test the filtrate for lead with a few drops of an alcoholic solution of sulfuric acid. If there is no precipitate showing that the lead is not in excess, repeat the analysis, using less fatty acid or more lead acetate. Wash the precipitate with 95 per cent alcohol until a sample of the washings diluted with water remains clear. Transfer and wash the precipitate from the filter back into the beaker, using about 100 cc. of 95 per cent alcohol. Add 0.5 cc. of acetic acid and heat to boiling. (The precipitate will slowly dissolve.) Allow to cool to room temperature and then place in the ice box as before. Filter and wash with 95 per cent alcohol as before. Transfer the precipitate into a 500 cc. separatory funnel by washing with ether. Add about 20 cc. of concentrated hydrochloric acid and shake until the lead soap is decomposed. Add some water and again agitate. After settling, draw off the aqueous layer. Wash the ether solution with water until the washings remain clear and then add a silver nitrate solution. Dehydrate the ether solution with 3-4 grams of anhydrous sodium sulfate and transfer it to a weighed Erlenmeyer flask. Distil off the ether, dry in an oven at about 110°C., and weigh. Determine the iodine number to ascertain whether more than an insignificant amount of unsaturated acids has been left with the saturated acids. The iodine number of the saturated acids fraction separated by this method from oils such as cottonseed, olive, peanut, soybean, etc., should not be more than 1 or 2.

The results reported by the collaborators are given in Tables 2 and 3.

DISCUSSION OF RESULTS.

When it is considered that extensive experience is necessary before the analyst can master the difficult technic of the lead-salt-ether method,

TABLE 2.

Collaborative results with the lead-salt-ether method.

ANALYST	SAMPLE NO.	SATURATED ACIDS AS DETERMINED	IODINE NO.	SATURATED ACIDS CORRECTED	UNSATURATED ACIDS AS DETERMINED	UNSATURATED ACIDS CORRECTED	IODINE NO. UNSATURATED ACIDS
		<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
C. A. Knuth	1	9.25 9.50	9.8 7.6	8.61 8.99	81.36 81.12	82.00 81.68	141.7 141.1
W. F. Baughman	1	10.10 9.90	5.5* 7.4*	9.70 9.40	82.70 82.90	83.10 83.40	137.3 136.9
W. D. Richardson	1		14.31 14.73	9.75 8.05		81.62 82.02	145.5 140.4
M. L. Sheely	1	8.50 8.30	4.3 8.3	8.15 7.70	77.00 84.00	77.30 84.10	103.3 121.0
C. A. Knuth	2	22.49 22.27	8.3 10.0	21.22 20.75	72.51 73.18	73.79 74.70	146.5 146.7
W. F. Baughman	2	21.70 21.60	3.8* 5.9*	21.10 20.07	73.2 72.8	73.70 73.70	144.8* 142.2*
W. D. Richardson	2		7.6 7.4	21.77 20.76		72.92 73.35	151.3 148.0
M. L. Sheely	2	24.00 22.40	6.0 9.1	22.30 20.50	66.30 72.70	68.00 74.60	95.4 105.2
C. A. Knuth	3	17.79 17.84	4.6 5.3	17.15 17.10	77.39 77.06	78.03 77.80	127.3 128.6
W. F. Baughman	3	17.70 18.10	3.3* 3.7*	17.20 17.60	77.50 75.96	78.00 76.40	120.6* 121.4*
W. D. Richardson	3		8.3 6.3	17.35 16.68		76.07 76.14	128.3 128.9
M. L. Sheely	3	17.20 18.00	5.3 7.1	16.30 16.90	75.30 72.70	76.20 73.90	97.2 119.2

* Iodine numbers determined by Hanus method; others by Wijs method.

the results in Table 2 with few exceptions show fairly good agreement, particularly in the case of the percentages of saturated acids; as was to be expected, the results with the more difficult determination of the unsaturated acids show a wider variation. It should be noted that in each case Baughman used the Hanus method for the determination of the iodine numbers of the unsaturated acids, while most of the other collaborators stated that they used the Wijs procedure, which gives somewhat higher values.

Table 3, which gives the percentages of the saturated acids by the Twitchell method as well as their iodine numbers, contains no column of results of saturated acids corrected for the quantity of unsaturated acids present as shown by their iodine numbers because this method is

TABLE 3.

Collaborative results with lead-salt-alcohol (Twitchell) method.

ANALYST	SAMPLE NO.	SATURATED ACIDS AS DETERMINED	IODINE NO. SATURATED ACIDS
C. A. Knuth	1	<i>per cent</i>	
		10.51	8.3
		10.47	9.4
		10.24	10.0
W. F. Baughman	1	8.60	3.8
W. D. Richardson	1	10.88	6.69
		10.93	6.0
M. L. Sheely	1	2.44	43.2
		2.69	59.4
C. A. Knuth	2	22.94	4.2
		23.13	4.7
		23.02	3.8
W. F. Baughman	2	20.60	4.0
W. D. Richardson	2	21.97	0.97
		21.36	0.95
M. L. Sheely	2	17.50	0.55
		19.40	2.40
C. A. Knuth	3	19.10	4.5
		18.73	5.1
		19.05	5.2
W. F. Baughman	3	16.10	1.1
W. D. Richardson	3	17.27	1.66
		17.84	1.55
M. L. Sheely	3	14.50	4.4
		12.90	2.6

supposed to give a saturated acid fraction containing so little unsaturated acid as to require no correction. The results in Table 3 show that in a number of instances the saturated acids have iodine numbers of 4 or more. Other analyses made at various times in the referee's laboratory by the Twitchell method confirm these results, although special effort was made in these cases to remove as completely as possible the unsaturated acids by washing the lead salts continuously for many hours with 95 per cent alcohol. Steger and Scheffers¹ have shown that the separation of the saturated from the unsaturated acids by the Twitchell method is greatly influenced by the nature and quantity of saturated acids present in an oil.

¹ *Rec. trav. chim.*, 1927, 46: 402.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the André-Cook method for the determination of acetyl value be made official.
- (2) That no further study be made at present of the Thomas-Yu and the modified Bellier methods for the approximate determination of peanut oil in the presence of olive oil.
- (3) That the study of the lead-salt-ether method be continued.
- (4) That the study of the Twitchell lead-salt-alcohol method for the determination of saturated fatty acids be discontinued.
- (5) That the "cold test" method as applied to salad oil be studied.

REPORT ON BAKING POWDER.

By L. H. BAILEY (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

During 1927 the work on baking powder centered largely on the problem of the quantitative separation of the phosphates. The task is a difficult one, and a satisfactory solution has not been found. However, recent publications of researches on the phosphates give encouragement to the hope that some day official methods will be available for separating and estimating the different forms of phosphates that are found in baking powders and baking acids.

The referee prepared for study a mixture of commercial monocalcium phosphate and commercial sodium acid pyrophosphate. After working for some time with this mixture and with its components, he devised a method of analysis which, with this particular sample, gave good results. The procedure was only tentative, however, to serve as a basis for a more comprehensive method, as it was recognized that it would not have universal application.

Samples were sent to collaborators with directions for determining the percentage of calcium in the mixture and calculating this value to monocalcium phosphate and also for determining total phosphorus pentoxide. The percentage of phosphorus pentoxide in the monocalcium phosphate was to be deducted from the total phosphorus pentoxide, and the remainder was to be calculated to sodium acid pyrophosphate. If only monocalcium phosphate, sodium acid pyrophosphate, and water were present, the total should be 100 per cent.

The collaborative results show only fair agreement. The great differences in moisture values are due, without doubt, to differences in the temperature at which the samples were dried. The monocalcium phosphate probably was the monohydrate and not the anhydrous, so that

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1927, 11: 78.

water of constitution was removed when the temperature exceeded 100°C., as it did in one case.

The collaborative results follow:

COLLABORATOR	H ₂ O per cent	CaH ₄ (PO ₄) ₂ per cent	Na ₂ H ₂ P ₂ O ₇ per cent	TOTAL per cent
Howard Adler Victor Chemical Works Chicago Heights, Ill.	0 35	49.09	44.40	93.84
Percy O'Meara Department of Agriculture Lansing, Mich.	0.70	50 30	47 70	98.70
Augustus H. Fiske Rumford Chemical Works Providence, R. I.	0.19	51 19	43.98	95.36
L. H. Bailey	3 56	49 18	47 44	100.18

It is recognized that commercial phosphates frequently are mixtures and not single substances, that all the calcium present may not be in the form of monocalcium phosphate, and that phosphates other than monocalcium and sodium acid may be present. Therefore, owing to its limited application, further study of the proposed method was not made.

Samuel J. Kiehl and his coworkers at Columbia University have recently published several papers on phosphates (see literature references). In one of these papers a method is presented for separating monometaphosphoric acid from orthophosphoric acid. Another paper treats of pyrophosphates. It is suggested that the next referee give consideration to these methods.

W. E. Stokes of the Royal Baking Powder Company suggested the use of caprylic alcohol to reduce the foam in certain tests, such as the determination of residual carbon dioxide. He states: "We add from one to three drops of caprylic alcohol to the baking powder in the flask and proceed in the usual manner". The referee tried this modification and found it satisfactory. The caprylic alcohol may also be used to reduce the foam in the method for the electrolytic determination of lead in baking powder.

One way to determine the percentage of sodium aluminum sulfate in a combination baking powder is to calculate from the aluminum present. Since more recent methods of determining alumina are found in the literature (see references) than the one given in *Methods of Analysis*, it is suggested that the next referee give consideration to this subject.

RECOMMENDATIONS¹.

It is recommended—

(1) That the modified gasometric method for the determination of total carbon dioxide² be adopted as official (final action).

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 78.

² *This Journal*, 1927, 10: 36.

(2) That the gasometric method for the determination of residual carbon dioxide¹ be amended by adding the sentence, "One to three drops of caprylic alcohol may be added to the baking powder in the decomposition flask to prevent foaming", and that the amended method be made official (final action).

(3) That study be continued on the separation and determination of the different forms of phosphates used as baking acids.

(4) That consideration be given to methods for the determination of alumina.

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(5) McNABB, WALLACE M.—An Accurate Method for the Determination of Phosphorus Pentoxide as Magnesium Ammonium Phosphate. *J. Am. Chem. Soc.*, 1927, **49**: 891-96.

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¹ *This Journal*, 1927, **10**: 36.

DRUG SECTION.

REPORT ON DRUGS.

By ARTHUR E. PAUL (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Referee*.

The results attained this year represent the efforts of thirteen associate referees and approximately forty-two collaborators. It is regretted that two associates were prevented by various circumstances from presenting any reports. However, it is felt that the work performed has resulted in satisfactory progress in the development of drug methods in this association.

Of the seventeen topics studied, six have been brought to a condition which, in the estimation of the associate referees and of the referee, warrants discontinuance. Eight new topics are now recommended, and it seems desirable that associate referees be appointed for their study.

The Associate Referee on Crude Drugs studied methods for fluidextract of ginger. Since, however, the subject of ginger extract used for food purposes is properly assigned to the Associate Referee on Flavoring Extracts, it would seem desirable to appoint an associate referee on ginger preparations, as explained later under the subject of crude drugs.

Comments by and specific recommendations of the referee will be made on the various topics that were studied this year.

It is recommended—

- (1) That the following topics be discontinued:

Cocaine,
Arsenicals,
Chaulmoogra Oil,
Pyramidon,
Silver Proteinates, and
Ipecac Alkaloids.

- (2) That the following topics be continued:

Crude Drugs,
Ether,
Alcohol in Drugs,
Chloroform and Carbon Tetrachloride,
Radioactivity in Drugs and Water,
Mercurials,
Microchemical Methods for Alkaloids,
Terpin Hydrate, and
Bioassay of Drugs.

- (3) That last year's recommendations on the following subjects be repeated, since no reports were received from associate referees.

Laxatives and Bitter Tonics and Santonin.

(4) That associate referees be appointed for the study of the following subjects:

Ephedra,
Ginger Preparations,
Pilocarpine in Tablets,
Thymol,
Menthol,
Bromides in the Presence of Chlorides,
Oil of Chenopodium, and
Sabadilla Assay.

(5) That the following comments and recommendations be considered in connection with the associate referee's report in each instance:

ALCOHOL IN DRUGS.

Considerable work was done during the two preceding years by the former associate referee, who studied the effect of a number of interfering substances. Some interesting data were submitted by him, although none of the methods was recommended for adoption.

The associate referee, this year, continued these studies. He also included some investigation as to whether it is necessary or desirable to vary the procedure for the actual determination of alcohol, in working on drugs, from those methods which are now included in *Methods of Analysis*. The results obtained indicate that this is not necessary if the sample is prepared according to the special details specified.

The studies this year include the interferences due to the presence of:

- | | |
|------------------------|-----------------------|
| 1. Iodine | 4 and 5. Formaldehyde |
| 2. Oil Emulsion | 6. Paraldehyde |
| 3. Chloroform Liniment | 7. Methyl Alcohol |

The associate referee made no recommendation, but the results reported on some of the items studied are encouraging. U. S. P. X includes directions for the determination of alcohol in the presence of iodine, essential oils, and other volatile substances, and therefore action with respect to these is unnecessary.

A study was also made of the quantitative estimation of ethyl alcohol in the presence of methyl alcohol. The method studied is an adaptation of the qualitative test for methyl alcohol in ethyl alcohol given in U. S. P. X; it depends on the far greater reactivity of methyl alcohol than ethyl alcohol with potassium permanganate. The distilled mixed alcohols are treated in the cold with this reagent, the excess is removed with oxalic acid, and the formaldehyde produced is determined by color comparison with Schiff's reagent. The results reported are promising, but the method should be further studied. In addition, the description of the method is based on the examination of a known mixture and

requires rewriting in order to make it applicable to samples of unknown composition.

The results of the other studies, while encouraging, were such as hardly to warrant adoption at this time.

Consideration was also given by the associate referee to the determination of ethyl and isopropyl alcohol when occurring together. He invited his collaborators to suggest suitable methods, but he received no responses. The Revision Committee of the U. S. P. has this determination under consideration and is now studying a procedure proposed by Dale and Simonds. It is suggested that the next associate referee communicate with the chairman of that committee, E. Fullerton Cook, 636 South Franklin Square, Philadelphia, Pa., for the purpose of deciding whether it is necessary or desirable to carry on any work on this topic, or whether a suitable method will be included in the next revision of the U. S. P.

The referee makes the following recommendations¹:

(1) That further study be made of the associate referee's suggested method for methyl and ethyl alcohol, and of any other available methods for this determination.

2. That special attention be devoted to methods for the determination of ethyl, isopropyl, and similar higher alcohols.

3. That the methods studied, other than those provided for in U. S. P. X, be again submitted to collaborative investigation.

ARSENICALS.

Two methods for the determination of arsenic are now tentative, and the associate referee explains the need for a third method. It seems necessary, however, that the proposed method be supplied with a suitable designation in order to differentiate it from the two older methods.

The associate referee's recommendations are approved, but it is suggested that the procedure he proposes be designated "Iron Cacodylate Method". This topic will then be considered closed.

COCAINE.

Two methods were studied by last year's associate referee, but they were not adopted by the association, because additional investigation was deemed desirable. A third method was proposed for study by the referee, a modification of a procedure used by the American Drug Manufacturers Association.

These three methods were studied collaboratively by the associate referee, and his results show practically no choice as to accuracy. The associate referee's two methods comprise merely two different ways of finishing the same determination, using the same sample. It seems, therefore, that they should be considered as one. This combination pro-

¹ For report of Subcommittee B and action of the association on drugs, see *This Journal*, 1928, 11: 72.

cedure has the advantage of providing a check determination; the A. D. M. A. method has the advantage of simplicity.

It is believed that both processes should be adopted, that a suitable designation should be assigned to each, and that the associate referee's directions should be slightly amended in order to combine his two methods.

(1) It is recommended that the two methods designated by the associate referee as "volumetric" and "gravimetric" be amended and consolidated into one. This method has been published¹.

(2) It is recommended that the A. D. M. A. method be slightly modified and designated the "single extraction method". This method has also been published¹.

(3) It is recommended that these two methods be tentatively adopted and that this topic be then considered closed.

CRUDE DRUGS.

Practically no work has been done on this topic in the past. This year Associate Referee Clevenger made an interesting study of methods for the examination of fluidextract of ginger. The results submitted are promising, but his conclusion is that the study of the proposed methods should be continued next year. This recommendation is approved.

Fluidextract of ginger is a U. S. P. product and is used in medicine. It must, therefore, be classed as a drug. On the other hand, so far as analytical methods are concerned, the product coincides with "Ginger extract" as listed in Circular 136² under Flavoring Extracts. Methods for the latter "extract" have in the past been studied by referees on flavoring extracts. Consideration of ginger extract is therefore probably included by the referee for that class of products.

Since from the standpoint of analysis the two products are similar, there may be duplication of effort and it would seem desirable for the association to decide which referee should study given preparations. In this connection it should be pointed out that while fluidextract of ginger is no doubt a drug, it can hardly be classed as a crude drug, but is, in fact, a pharmaceutical preparation. Since no referee has been appointed on the broad subject of pharmaceuticals, it would seem desirable next year to appoint a special referee on ginger products.

If this plan is approved, it will be necessary to have it understood that the Referees on Flavoring Extracts and on Crude Drugs, if appointed, will be relieved of any consideration of ginger products.

CHLOROFORM AND CARBON TETRACHLORIDE.

In 1926 a method for chloroform and carbon tetrachloride devised

¹ *This Journal*, 1928, 11: 49.

² U. S. Dept. Agr. Circ. 136, 1919.

by Moraw was tentatively adopted¹. It involves heating with alcoholic potassium hydroxide in pressure bottles. The need for using such bottles was questioned by some collaborators, and it was the intention of this year's associate referee to reinvestigate this feature. He, however, introduced some new features which, while interesting, failed to reach the accuracy obtained by Moraw and his collaborators.

It is therefore recommended that the present method be retained as tentative until an equally accurate but simpler procedure is worked out.

Last year it was also suggested by the referee, and recommended by Committee B, that the details proposed by Moraw for the determination of chloroform and carbon tetrachloride in various mixtures be further studied, but the associate referee found it impossible to perform any work on this feature during the present year.

It is recommended, therefore, that this topic be continued for another year with a view, especially, to the determination in mixtures.

CHAULMOOGRA OIL.

In view of the associate referee's report, it is recommended that this topic be discontinued.

IPECAC ALKALOIDS.

The associate referee recommends adoption, tentatively, of the methods described by him as No. 1 and No. 2. In view of the satisfactory agreement of results reported by collaborators, approval of these recommendations seems desirable.

In addition to the recommendations of the associate referee, it is now suggested that this topic be considered closed.

RADIOACTIVITY IN DRUGS AND WATERS.

The associate referee continued the comprehensive programs that he started several years ago and carried the work forward by preparing a series of authentic radium samples for subsequent collaborative study.

LAXATIVES AND BITTER TONICS.

No report was received from the associate referee. Last year's recommendations should be repeated.

MERCURIALS.

U. S. Pharmacopeia X includes an electrolytic assay for mercury, as well as three chemical procedures. These, however, require adjustment in order to be applicable to tablets, and it was the task of the associate referee to study them, suitably amended, as well as any other available procedures.

¹ *This Journal*, 1927, 10: 45, 68.

Last year the associate referee pointed out certain reasons why it is desirable to have available a chemical method for determining mercury in calomel tablets. The electrolytic method, it is assumed, is satisfactory and was not studied. The associate referee this year studied four chemical methods and from the results obtained decided upon a modification of the U. S. P. iodine method, the modification being made for the purpose of adaptation for calomel tablet assay.

The associate referee's recommendation for tentative adoption is approved. It is also recommended that this topic be continued for the purpose of studying other mercury compounds.

PYRAMIDON.

During 1924 two quantitative methods were studied by the associate referee, who recommended them for further study. Committee B, however, recommended tentative adoption, and this was approved by the association. Nevertheless a further modification was proposed by Associate Referee Rabak in 1925 and repeated in 1926.

The two present tentative methods are alike in principle; they differ only in the nature of the final compound weighed. The modification studied by Rabak does not affect the principle—it merely substitutes ammonia for the fixed alkali in the tentative extractions.

This modification was studied this year by Associate Referee Elliott and a considerable number of collaborators in comparison with the two tentative procedures. The results show a decided preference for the latest modification because the figures reported throughout show less variation than either of the now tentative methods.

It is therefore recommended that in lieu of the two present tentative methods the modification described by Rabak¹ and studied this year by Elliott and his collaborators be adopted as a tentative method and that this subject be discontinued.

MICROCHEMICAL METHODS.

The recommendations of the associate referee are approved, and it is recommended that this topic be continued for the study of other alkaloids.

SILVER PROTEINATES.

With the tentative adoption of the yeast method for ionizable silver, this topic was considered closed last year with one exception—the question of alkalinity and acidity. The associate referee attempted to devise a potentiometric method by examination of dialyzed solution, but un-

¹ *This Journal*, 1926, 9: 309.

fortunately he failed to obtain the necessary break in the curve. He then devoted his attention to the titration method originally used by Eaton¹. While the number of results reported by the associate referee is not large, it is believed that they, together with those reported by Eaton, warrant tentative adoption of the method. Such action is recommended by the associate referee and it is believed should be approved, with the understanding, however, that the method be retained as tentative and that it be not made official in the regular course of procedure.

It is hoped that other investigators will give some study to the possible development of a satisfactory potentiometric procedure.

TERPIN HYDRATE.

This topic is relatively new, so far as official study by this association is concerned. However, an article by A. G. Murray², in which a method was proposed, formed the basis for the associate referee's investigation. The results reported by him are encouraging, but he recommends that the method and also the interfering chloroform-soluble substances be studied further. Also, that the use of a mechanical extractor for this determination be investigated. These recommendations are approved.

SANTONIN.

No report was received from the associate referee. Last year's recommendations should be repeated.

ETHER.

The desirability of having satisfactory methods for the determination of ether in the presence of alcohol and other interfering substances has been pointed out previously in the proceedings of this association. Some headway was made in connection with this difficult topic by the associate referee this year, but no recommendation for adoption was made. As was proposed, it is recommended that this topic be continued for further study of the method.

BIOASSAY OF DRUGS.

While the cat-eye method for mydriatics was recommended for tentative adoption last year, this action was not taken by the association. The associate referee's two recommendations, therefore, cannot be adopted as proposed by him. In lieu thereof it is suggested that the cat-eye method be adopted tentatively for mydriatics and myotics.

¹ *This Journal*, 1926, 9: 312.

² *Ibid.*, 1927, 10: 257.

REPORT ON ALCOHOL IN DRUGS.

By CHARLES D. HOWARD¹ (Division of Chemistry, State Board of Health, Concord, N. H.), *Associate Referee*.

During 1925 and 1926 some progress was made in the development of procedures for use in the determination of alcohol in the presence of various interfering substances. These procedures and the results obtained were published in the reports of the associate referee², with comments and recommendations by the referee³.

The associate referee for 1926 recommended that the investigation be extended to include the estimation of alcohol in the presence of paraldehyde and proposed a method. Study of a colorimetric procedure for alcohol, of methods for the estimation of isopropyl alcohol, and of the effect of redistillation in cases where the quantity of alcohol in the sample is small was also recommended.

It seemed desirable to devote the work this year to a collaborative study of these procedures. Instructions were prepared and sent to the 25 chemists whose names had been furnished, as well as to a number of others. Unfortunately, but five made reports; nor did it prove feasible, because of pressure of official duties, for the associate referee to carry through any of the analytical work.

Concerning the instructions given, the text of which follows, it should be appreciated, in connection with consideration of the results reported, that these follow quite closely the specifications as outlined by the referee in 1925 (page 268), and those of the associate in 1926, the amplifications here given being suggestive as to details of operation as worked out in a preliminary way by the present associate. The only additional procedure incorporated was that with respect to substantial quantities of formaldehyde (5).

The following collaborators reported results:

E. M. Bailey and C. E. Shepard, Agricultural Experiment Station, New Haven, Conn.

Marion Brimston, University of Washington, Seattle, Wash.

E. R. Tobey, Agricultural Experiment Station, Orono, Me.

Leonard Feldstein, U. S. Food, Drug and Insecticide Division, Denver, Colo.

L. B. Rhodes, State Department of Agriculture, Raleigh, N. C.

None of the five collaborators to report did anything with isopropyl alcohol (8). The specifications for (2) and (3) were purposely made somewhat difficult in order to simulate problems frequently arising in practice. The procedure for paraldehyde (6) is precisely as given by the associate referee last year.

¹ Presented by C. K. Glycart.

² *This Journal*, 1926, 9: 282; 1927, 10: 342.

³ *Ibid.*, 1926, 9: 267; 1927, 10: 335.

INSTRUCTIONS TO COLLABORATORS.

Express all results as percentage by volume, based upon the final procedure as given in 3 and 4, page 361, of *Methods of Analysis*. As there may be some difficulty from foaming in one or two of the distillations, use long-necked, 500 cc., Kjeldahl flasks for the distillation. (They should be connected to spiral-type, upright condensers of a good length by the usual spray trap.)

Take for distillation a uniform volume of 100 cc., measured at 20°C., dilute to about 150 cc., and distil to nearly 100 cc., making to the mark at 20°C. (Not less than 30 minutes should be consumed by the distillation after boiling commences.) Make all determinations in duplicate, and, if possible, in triplicate.

As a basis for the work, prepare a quantity of diluted alcohol of a concentration not exceeding 15 per cent by volume and, for the sake of uniformity, preferably not less than 12 per cent, storing at a temperature under 20°C. Estimate the percentage as accurately as possible and run a repeat prior to each of the estimations called for in case of any substantial interval between or any other disturbing conditions. In carrying out the determinations, measure 100 cc. at 20°C. and introduce the substances to be referred to, adding sufficient water to bring the volume to about 150 cc. before distilling to 100 cc.

1. *Iodine, 2 per cent.*—Add 2 grams of iodine dissolved in a little potassium iodide solution. Add a sufficient quantity of zinc dust and distil when reduction is complete.

2. *Oil emulsion.*—Rub up 2 cc. of any volatile oil conveniently at hand (preferably one of fairly high density, such as clove) with 0.25 gram of gum tragacanth or gum arabic, add about 25 cc. of water, transfer to a bottle, and shake vigorously. Add this emulsion to the 100 cc. alcohol contained in a 150 cc. separatory funnel, saturate with sodium chloride, and shake out with about 20 cc. of petroleum ether, allowing sufficient time for a sharp separation. Transfer the watery layer to a second separatory funnel and again shake out. Wash the combined ether extracts with 10 cc. of sodium chloride saturated water, add the latter to the alcohol-water solution, make to a volume of 150 cc., and distil as usual.

(In the presence of gums it will usually be found impossible to remove all trace of oil by this treatment, even with a third extraction. Therefore it will be of interest to repeat the extraction upon a fresh portion and re-extract the distillate as in (2), noting any differences in percentage.)

3. *Camphor, soap, chloroform, and oil mixture.*—This represents essentially the problem involved by the estimation of alcohol in camphor liniment. Prepare a quantity of alcohol-free chloroform by washing twice with five volumes of water. Rub up in a dry mortar 1 gram each of powdered soap and camphor with 1 cc. of a volatile oil and 10 cc. of chloroform (washed). Transfer to a shaking bottle with about 35 cc. of water, agitate vigorously, add to the 100 cc. diluted alcohol, and distil direct after adding 3 grams of tannic acid to prevent foaming. For such purpose this quantity will usually be found sufficient. Saturate the distillate with sodium chloride and proceed with the extraction as in (2).

4. *Formaldehyde, 1 per cent.*—Add 2.5 cc. of commercial formalin and oxidize by adding 50 cc. of hydrogen peroxide U. S. P. and an excess of saturated sodium hydroxide solution, refluxing over a water bath until effervescence ceases. Before distilling, test for completeness of oxidation by adding to the cooled liquid a few crystals of phloroglucin, also making certain that the reaction is still strongly alkaline by adding a small quantity of dry phenolphthalein.

5. *Formaldehyde, 20 per cent.*—This is essentially the problem commonly involved by the estimation of alcohol in embalming fluids, which contain 15–30 per cent of formaldehyde. Transfer the 100 cc. diluted alcohol to the distilling flask; add 5 cc. of 50 per cent sodium hydroxide solution, then 10 grams of potassium or sodium cyanide;

and rinse down the walls with 50 cc. of commercial formalin, distilling to 100 cc. (CAUTION.—Without the addition of sodium hydroxide some hydrocyanic acid, or a cyanogen compound, will distil over, while with a sufficient excess of alkali, considerable ammonia is evolved. CAUTION.—Potassium cyanide brought in contact with concentrated formalin results in active warming up.) To oxidize the small quantity of formaldehyde not held back by this procedure, treat the distillate with hydrogen peroxide as directed in (4), finally rendering acid with sulfuric acid before redistilling, in order to hold back any ammonia.

6. *Paraldehyde, 3 per cent.*—Dissolve 3 cc. of paraldehyde in about 30 cc. of water, add to the 100 cc. diluted alcohol, and oxidize by adding an excess (?) of Tollen's reagent and refluxing over a water bath for one-half hour. Cool and distil as usual. Prepare Tollen's reagent by first making a 10 per cent solution of silver nitrate in equal parts of water and ammonia (sp. gr. 0.90); to prepare the reagent for use mix equal parts of this solution and 10 per cent aqueous sodium hydroxide solution. (CAUTION.—Do not mix with the soda solution until needed, as on long standing such a mixture deposits a highly explosive black precipitate.)

7. *Methyl alcohol, 1 per cent.*—Dilute a pure grade of 95 per cent methyl alcohol to about 10 per cent, accurately estimate the percentage of methyl alcohol therein (using the same tables and procedure as for ethyl), and to 100 cc. of the regular diluted alcohol add the equivalent of about 1 cc. absolute methyl alcohol, accurately measured so that the exact quantity present is known. Distil to 100 cc. and estimate the percentage of mixed alcohols as usual. So dilute the distillate as to obtain a solution containing 0.1 per cent methyl alcohol, accurately noting the dilution. Prepare a standard containing in 100 cc. exactly 0.1 cc. absolute methyl alcohol and about 1 cc. ethyl alcohol. To 2 cc. each of the standard and of the diluted distillate add 2 cc. of 3 per cent permanganate solution and 0.5 cc. of sirupy phosphoric acid. Allow to stand 5 minutes, decolorize by adding a slight excess (1 cc. is sufficient) of 10 per cent oxalic acid solution and let stand a few moments until solution is a transparent brown. Now add 5 cc. of dilute sulfuric acid (1-3) and 5 cc. of fuchsin-sulfurous acid solution (T. S., U. S. P. X, page 488). Dilute to a volume of 25 cc., allow to stand about 10 minutes, and compare the colors in a standard colorimeter. From the data compute the percentage of methyl alcohol in the sample. To estimate the ethyl alcohol, deduct the methyl alcohol thus found from the total alcohol in the distillate.

8. *Isopropyl alcohol.*—Report the details of any procedure you may be using for the estimation of isopropyl alcohol in the presence of alcohol.

9. *Small quantities of alcohol.*—Make up a known dilution containing about 0.50 per cent of alcohol. Note any difference in percentage as a result of the single distillation to 100 cc. and redistilling to 50 cc.

COMMENTS BY COLLABORATORS.

E. M. Bailey and C. E. Shepard.—*Alcohol.*—The stock solution was prepared by taking 650 cc. of absolute alcohol and diluting to 5000 cc. with water. All aliquots were taken at 20°C. Tests indicated no change in the strength of the stock solution during the period of the experimental work, July 22 to August 4. During most of the time the room temperature ranged from 80° to 90°F., with high humidities. No attempt was made to cool the receiving flask.

1. *Iodine.*—This method is satisfactory.

2. *Volatile oil.*—With method as prescribed the recovery is about 0.3 per cent low and the first distillate always cloudy, with an odor of volatile oil. To overcome this condition it seemed desirable to (1) shake out with a mixture of petroleum ether and chloroform (2 : 1) and also (2) to wash four times with saturated salt solution instead of once. These modifications did not improve recoveries and did not produce first

distillates which were clear. More washings with salt solution would seem, however, to be advisable as a precaution. The method as prescribed is fairly satisfactory, but recoveries are a little low.

3. *Camphor, soap, chloroform, and oil mixture*.—Distillates clear; no odor of chloroform or of clove. More washings with saturated salt solution (about four) are necessary to afford recoveries within about 0.2 per cent of the correct values.

4. *Formaldehyde, 1 per cent*.—Although the recoveries appear to be correct the distillate is not a mixture of alcohol and water. There is probably formaldehyde and/or methyl alcohol in the distillate.

5. *Formaldehyde, 20 per cent*.—Results unsatisfactory.

6. *Paraldehyde, 3 per cent*.—Odor of paraldehyde very marked in each distillate. There seemed to be no further reduction of silver after adding 7 cc. of Tollen's reagent. Possibly less than 7 cc. should have been tried. The results are unsatisfactory, but we have no suggestions for improvement at this time.

7. *Methyl alcohol, 1 per cent*.—The time allowed before taking the colorimetric readings is much short of that necessary for the maximum color to develop. Furthermore, the percentage of total alcohol is not the same in the standard as in the experimental mixtures which are compared—in this case 1.10 per cent and 1.41 per cent, respectively. These items are believed to be the sources of error in the recoveries.

The method we used is an adaptation of the U. S. P. method and is carried out as follows: Take 5 cc. of distillate in a 100 cc. volumetric flask, add 1 cc. of dilute phosphoric acid (1 part of 85 per cent phosphoric acid and 1 part water), mix, add 2 cc. of 3 per cent potassium permanganate solution, and allow to stand 10 minutes. Add 1 cc. of 10 per cent oxalic acid solution and when the solution has become transparent (in about 2 minutes), add 5 cc. of dilute sulfuric acid (1 part sulfuric acid to 3 parts of water). When the solution is completely decolorized, add 5 cc. of fuchsin-sulfurous acid, mix, and allow to stand 2 hours.

In making up solutions to contain 0.1 per cent methyl alcohol, enough ethyl alcohol is added to make approximately 5 per cent of total alcohol. This seems to be important. It is also important that the unknown solution be compared with a known standard that approaches it closely in color, otherwise the calculated values may be quite inaccurate.

8. *Isopropyl alcohol*.—We use only the U. S. P. qualitative tests and have no methods to suggest for quantitative work.

9. *Small quantities of alcohol*.—There appears to be no advantage in redistillation to 50 cc.

Leonard Feldstein.—Considerable difficulty was experienced in oxidizing all the formaldehyde in No. 4 and No. 5¹. Possibly sodium peroxide could be used to better advantage than hydrogen peroxide to oxidize the formaldehyde. It was practically impossible to get complete oxidation with the quantity of hydrogen peroxide prescribed by the method. Formaldehyde could be detected in the distillate by odor. The formaldehyde used was commercial and the label stated it contained 6-14 per cent of methyl alcohol.

The destruction of the paraldehyde also presented difficulties. Increasing quantities of Tollen's reagent were used in several determinations, and in the results submitted the odor of paraldehyde was still detected, which accounts for the low alcohol.

In the determination of methyl alcohol it was necessary to allow the diluted fuchsin-alcohol solution to stand 20 minutes before a sufficient coloration was obtained.

DISCUSSION.

With the iodine formula one collaborator obtained practically the theoretical recovery; one, results but slightly low; two, results only

¹ This criticism is inapplicable to No. 5. See comments by associate referee.

slightly in excess, while those reported by the fifth were of varying degrees of lowness. On the whole it is believed that this procedure should be regarded as entirely satisfactory.

Three collaborators obtained results but 0.3–0.4 per cent low for No. 2, oil emulsion, but those of the fifth collaborator were far under. It appears that while re-extraction of the distillate and subsequent distillation serve to remove the small quantity of oil going over into the first distillate, there is a tendency to lose some alcohol. Further washing of the original ether extract with salt solution seems to be advisable.

On No. 3—camphor, oil, soap, and chloroform mixture—all three collaborators reporting obtained materially lower recoveries by the procedure directed, but by washing four times with salt solution instead of once, one analyst obtained a substantially larger yield. It would appear that in none of these cases involving an ether extraction is a single washing with salt solution sufficient.

With the 1 per cent formaldehyde formula one analyst obtained results somewhat low; one, results only slightly in excess; another, in greater excess, while those of the fourth were close to theory, from specific gravity, but refractometrically the presence of some methyl alcohol derived from the formaldehyde was indicated. Concerning the criticism of one analyst relative to failure to obtain complete oxidation with hydrogen peroxide, it may be pointed out that except that the volume of fluid to which the peroxide is added (allowing for the necessary small quantity of strong alkali solution) is about 110 cc., instead of about 65 cc., the method as prescribed is identical with that official in the United States Pharmacopeia for the determination of formaldehyde, and which, long employed for this purpose, is presumed to give complete oxidation. Possibly an insufficient quantity of alkali was used. Completeness of oxidation is readily determined by testing with phloroglucin or similar reagent. While the presence of more or less methyl alcohol in ordinary formaldehyde solution is always to be expected, and in consequence results in excess of theory are to be anticipated, yet on the other hand unless care be observed, some loss of alcohol may result owing to imperfect condensation or to expulsion with the hydrogen.

Although two of the four analysts alluded to the presence of methyl alcohol in the formaldehyde used, none of these, apparently, adopted the simple expedient of making a correction based upon the result of a determination in blank.

No. 5, formaldehyde 20 per cent.—Considering the difficulty of this problem, the results reported by one collaborator are surprisingly good. Some of the operators were handicapped through the use of a formaldehyde with a substantial methanol content. According to Holleman¹,

¹ Text-book of Organic Chemistry, 4th Eng. ed., 1913, p. 142.

Molinari¹, Richter², and others, some methanol results from the action of alkali on formaldehyde. Whether this is substantial under the conditions here prescribed remains to be determined. With concentrated alkali¹ only formic acid and nascent hydrogen are said to result. Magnesium oxide³ has no action, but barium hydroxide causes some formation of methanol—only slight, however—because of the prompt neutralization of this base. Some attempt has been made by the referee to apply a procedure along these lines to the analysis of embalming fluids, for which a method in this connection is desirable, and the results of a preliminary investigation with the cyanide-alkali process as here described had seemed promising. A procedure mentioned by Molinari⁴ for the determination of methanol in formaldehyde consists in distillation of this mixture, largely diluted with water, with an excess of ammonia, it being claimed that the distillate contains only negligible traces of formaldehyde.

All results reported by the three analysts for the paraldehyde formula were substantially low, although those obtained by the referee in 1926 were excellent. The reason for the difficulty at this time is not clear. In two cases, at least, it is obvious that the paraldehyde removal was far from complete. Incidentally, there appears to be considerable uncertainty concerning what constitutes "an excess" of the silver reagent. This procedure should have further investigation before it is again presented for collaborative work.

With the procedure for the colorimetric estimation of small amounts of methyl alcohol two analysts reported results equivalent, or close, to theory, while the other two got results that were somewhat excessive. Both of the latter report the need of longer standing for color development, one advising 20 minutes, the other 2 hours.

This matter has been the subject of considerable study by Bailey, in whose laboratory the colorimetric procedure has been employed for this purpose for some time. Because of the finding that it is necessary, for accurate results, to have the quantities of methyl alcohol in the unknown and in the standard as compared the same within very narrow limits, this collaborator inclines to the view that the colorimeter is of dubious applicability. He prefers to make the comparisons against a series of prepared standards involving very small intervals.

Bailey also finds that the intensity of color by the U. S. P. test does not progress in simple proportion to increasing percentages of methyl alcohol. In practice he distils 25 cc. to 100 cc., treats 10 cc. of the distillate with saturated salt solution, shakes out with petroleum ether, washes the ether twice with salt solution (to remove substances which

¹ General and Industrial Chemistry, 2nd ed., Organic, Part 1, p. 248.

² Organic Chemistry, 2nd Am. ed., 1892, p. 192.

³ Watts' Dictionary of Chemistry, 1919, vol. 2, p. 569.

⁴ General and Industrial Chemistry, 2nd ed., Organic, Part 1, p. 247; Allen's Commercial Organic Analysis, 5th ed., vol. 1, p. 325.

Collaborative results on the estimation of alcohol
(Figures express percentages of

FORMULA	E. M. BAILEY AND C. E. SHEPARD			MARION BRIMSTON	
	Present	Recovered		Present	Recovered
		From specific gravity	From refractometer		
1. Iodine, 2%	13.08	13.10 13.09	13.07 13.07	13.88	13.38 13.62 13.80 13.48
2. Oil emulsion (clove, 1.33%)	13.08	12.82 ⁴ 12.84 ⁴	12.92 12.99	13.88	12.88 13.20 13.03 12.88
2. do, with re-extraction of distillate	13.08	12.78 ⁸ 12.73 ⁸	12.78 12.76
3. Camphor, soap, chloroform, and oil mixture	13.08	12.28 11.94	12.25 11.74	13.82	12.73 12.97 13.14 12.86
3. do, washing ether four times with NaCl solution instead of once	13.08	12.85 12.85	12.87 12.84
4. Formaldehyde, 1%	13.08	12.92 13.20 13.13 13.20	12.69 12.95 12.82 12.82	13.82	13.38 13.40 13.24 13.61
5. Formaldehyde, 20%	Results	unsatisfactory		13.82 14.70	13.60 15.14 15.48 14.73
6. Paraldehyde, 3%	13.08	11.00 ⁶ 11.08 ⁷	17.25 17.32	14.70	12.88 13.09 13.19 13.25
7. Methanol, 1%	m 1.00 e 13.08 14.08	total 14.10 14.08 methyl: 1.22†	total 13.40 13.37 ethyl: 12.86	m 0.92 e 14.70 15.62	m 0.92 0.93 e 13.83 13.85 14.77
7. do, as modified	do	methyl: 1.00 1.00	ethyl: 13.10 13.08
9. Small quantities of alcohol	0.54	0.54 0.54	0.51 0.51	0.66 0.54	0.46 0.48 0.47 0.45
9. do, redistilling to 50 cc.	0.54	0.52 0.52	0.52 0.52

in the presence of interfering substances.

alcohol by volume at 20°C.)

E. R. TOBEY		LEONARD FELDSTEIN		L. B. RHODES	
Present	Recovered	Present	Recovered	Present	Recovered
14.56	14.40 ^{1*} 14.41 ² 14.35 ³	14.09 14.20	14.20 14.25	14.43 ¹	14.54 14.54
14.56	14.20 14.25 14.49	14.09 14.20	13.65 13.78	14.43 ²	14.05 13.89
14.56	14.08 14.08
		14.43 ³	13.73 13.73
	
		14.09 14.20	14.35 14.31	14.43 ⁴	14.78 14.91
		14.09 14.20	24.15 21.95
		14.09 14.20	12.45 12.90
		m 1.00 e 14.09	m 1.15 e 14.06	m 1.00 ⁷ e 15.62	m 1.03 m 1.08 e 14.49 e 15.34
		15.09	15.21	16.62	16.57-16.37

* (1) Distilling 20 minutes; (2) distilling 35 minutes; (3) distilling 30 minutes; (4) distillates cloudy, odor of oil of cloves; (5) distillates nearly clear, odor none, or trace only; (6) using 7 cc. Tollen's reagent; (7) using 13 cc. Tollen's reagent.

† Average of 3 results; range 1.07 to 1.34 %.

might give false color reactions), then makes to 25 cc. after adding sufficient ethyl alcohol to make this dilution contain 5 per cent. Five cubic centimeters of this solution is taken for the oxidation, the color being compared against a series of standard tubes similarly prepared to contain 5 per cent of total alcohol.

If any quantitative procedure for isopropyl alcohol has ever been proposed, the referee has no knowledge concerning it. For qualitative purposes the test with Denige's reagent, as given in U. S. P. X (and which also serves admirably for acetone in the same test portion), leaves nothing to be desired. Possibly this test is susceptible to development in a quantitative way. The comparatively high refractometric value of isopropyl alcohol has suggested to the referee a possibility that this principle might find application in this connection.

In the case of small quantities of alcohol one collaborator reported excellent results from a single distillation to 100 cc., while the other experienced some loss. There would appear to be no advantage, with reasonably careful work, in a redistillation to a smaller volume in cases where approximately as much as one-half per cent is present. There is, however, ground for a belief that where the quantity is materially smaller than this, the redistillation to a smaller volume should be practised.

It is obvious that there is need of further study of this important subject. It is recommended¹ that for the work of the coming year the referee modify the procedures in harmony with the comments and suggestions given in this report, and that they be again presented for collaborative study.

REPORT ON ARSENICALS.

ARSENIC IN CACODYLATES.

By H. WALES (Drug Control Laboratory, Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

The A. O. A. C. studies on arsenic for 1925 and 1926 were confined to obtaining arsenic in a form suitable to be determined by one of the accepted methods. The 1925 method for the determination of arsenic in sodium cacodylate² consisted in the destruction of organic matter with sulfuric acid and potassium sulfate in the presence of starch and subsequent titration of the arsenic with iodine. The 1926 method was devised for the separation of iron and arsenic in medicinal products³. After the organic matter was destroyed with a sulfuric-nitric acid mixture and the arsenic was distilled as the trichloride it could be estimated by any of

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 72.

² *This Journal*, 1926, 9: 286.

³ *Ibid.*, 1927, 10: 343.

the usual methods, although only titrations with iodine and with bromate were considered.

The 1926 method was found to be satisfactory except when the arsenic was present as a cacodylate or methylarsenate. Then it was found necessary to use the sulfuric acid-potassium sulfate method for the destruction of the organic matter before distilling as arsenic trichloride. It should be mentioned that this mixture is not satisfactory for Blaud and arsenic preparations, for which sulfuric and nitric acids should be used.

This year it was thought advisable to study a combination of the 1925 and 1926 methods for the determination of arsenic in ferric cacodylate, ferric methylarsenate and mixtures of cacodylates and methylarsenates with iron compounds. Inasmuch as neither of the iron salts of known purity could be obtained, a sample of sodium cacodylate U. S. P. mixed with ferric sulfate was assumed to react in the same manner as ferric cacodylate. For purposes of comparison, anhydrous sodium cacodylate was determined by the following methods, the U. S. P. product being used: I. Drying at 120°C.; II. U. S. P. X assay; III. A. O. A. C. 1925 assay; IV. A. O. A. C. 1925-6 assays combined; V. Same as IV after the addition of iron.

Method IV (combination of 1925 and 1926 A. O. A. C. Methods).

Transfer a suitable quantity of the sample (0.2 gram, if possible) to a Kjeldahl flask and add 10 grams of potassium sulfate, 0.3 gram of starch, and 20 cc. of concentrated sulfuric acid. Digest over a low flame until frothing has ceased and then continue digestion over a slightly higher flame until colorless. Cool, and add 20 cc. of water. Dry the neck of the flask over a small flame; cool the contents; add 30 grams of sodium chloride, 5 grams of ferrous sulfate, 1 gram of sodium bromide, and 25 cc. of strong hydrochloric acid; and distil as directed in the 1925 method for the determination of arsenic in iron-arsenic pills.

Results of analyses obtained by the various methods are as follows:

METHOD	SODIUM CACODYLATE ANHYDROUS	
	F. C. Synkovich	H. Wales
	<i>per cent</i>	<i>per cent</i>
Dried at 120°C.....	75.32, 75.43	75.5, 75.3, 75.5
U. S. P. X.....	73.8, 73.8	73.7
A. O. A. C. 1925.....	73.92, 74.06	73.3, 73.1
Combined 1925-1926 methods.....	74.68, 74.30	73.7, 74.3, 74.4
Same after addition of $\text{Fe}_2(\text{SO}_4)_3$	74.32, 74.44	73.7, 73.7

The associate referee is of the opinion that the methods proposed for the assay of arsenic in sodium cacodylate and iron-arsenic pills are accurate, and therefore he recommends¹ that they be adopted as official methods, the modification previously described—which is essential when both iron and cacodylates (or methyl arsenates) are present—being included.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 72. See p. 48 for methods as adopted.

It has been the experience of the associate referee that the above methods can be applied to all types of medicinal arsenic preparations with which he has come in contact, with the possible exception of preparations containing large proportions of glycerine.

It is recommended that no further work on arsenicals be conducted by the A. O. A. C. at the present time.

REPORT ON COCAINE.

By ELGAR O. EATON¹ (U. S. Food, Drug and Insecticide Inspection Station, San Francisco, Calif.), *Associate Referee*.

Last year the associate referee submitted to collaborators a volumetric and a gravimetric method, with samples for analysis and study. Results were received from four collaborators. The proposed volumetric method gave fair checks and a good average and was recommended for adoption as a tentative method. The so-called gravimetric method consists of splitting off the benzoic acid radical and determining it by weight and then calculating to cocaine. This part of the method was not so satisfactory, and further work was recommended in order to clarify details of the method.

The collaborators reported this year on the following proposed methods: titration in a wet way, no evaporation; hydrolysis and weighing of benzoic acid (some also titrated the acid); and an amended method of the American Drug Manufacturers Association.

These methods have been published².

The following results were obtained by the collaborators on a 20 per cent cocaine hydrochloride and lactose mixture:

ANALYST	VOLUMETRIC per cent	GRAVIMETRIC per cent	TITRATION (OF GRAVIMETRIC)	A. D. M. A. per cent
Louis G. Petree	19.6	19.7	19.7
	19.7	19.7		19.8
		19.5		19.6
Frank C. Synkovich	18.8	18.9	18.7	18.6
	18.9	19.2	18.9	18.7
C. K. Glycart	18.6	18.2	18.6
	19.3	18.4		18.5
H. Wales	19.0	18.8	19.3
	19.1	18.9		19.4
Alfred W. Hanson	19.2	18.8	19.5
	19.4	19.0		19.6
	19.5			
Elgar O. Eaton	19.5	19.6
	19.5			19.6
L. E. Warren	19.3	19.2	19.3
	19.4	18.9		18.9
Average	19.3	19.0	18.8	19.3
Recovery (per cent)	96.5	95.0	94.0	96.5

¹ Presented by H. R. Watkins.

² *This Journal*, 1928, 11: 49.

COMMENTS BY COLLABORATORS.

Louis G. Petree.—The first extraction with ether from the ammoniacal solution by the A. D. M. A. method is likely to be hindered by a troublesome emulsion. This difficulty was not observed when sodium bicarbonate and petroleum ether were employed as in the proposed volumetric method. I think the quantity of alcohol used for obtaining solution of the residue in the A. D. M. A. method should be stated, inasmuch as the titration may be influenced thereby.

Frank C. Synkovich.—I prefer the volumetric method to the A. D. M. A. because in the latter there is some difficulty in dissolving the residue from the ether extract without employing heat.

C. K. Glycart.—(1) The use of petrolic ether has a theoretical advantage over ethyl ether as a solvent because less water is dissolved in petrolic ether, and therefore there is less chance of alkaline bicarbonate solution being entrained. However, the manipulation with the petrolic ether-separatory funnel extraction was found difficult for quantitative accuracy. Losses occurred when the stopper of the separatory funnel was removed in order to release the great pressure due to expansion of this solvent. (2) The determination of the benzoate radical would be a valuable method for checking the quantity of this important alkaloid especially in mixtures. (3) The usual method of extraction for alkaloids when applied to cocaine gives good results. At the Chicago Laboratory chloroform is used because it is the best solvent for cocaine, also less water of condensation results when evaporating spontaneously. One determination was performed in this manner, and the result was 18.82 per cent.

H. Wales.—I do not believe that you have stressed the need for slow evaporation sufficiently in the gravimetric method. The results reported were obtained by allowing the chloroform to evaporate in a closed compartment of the desk. Two other samples evaporated on a window ledge on a hot day, when there was only a slight breeze, gave results about 1 per cent lower than those reported. There would seem to be no doubt that these samples were evaporated spontaneously.

Alfred W. Hanson.—The Eaton method gives good results on the sample submitted. If the results are as accurate for all concentrations of the alkaloid I believe it will prove valuable. In assaying cocaine hydrochloride the work should be completed as soon as possible after making the solution alkaline.

CONCLUSIONS.

It would appear that accurate results with this easily hydrolyzed alkaloid are rather difficult to obtain even in the hands of experienced analysts. The comments of the collaborators indicate some of the reasons for variations in results. The following seem to be the principal ones: (1) The solutions of the alkaloid should be worked rapidly; (2) the usual careful quantitative precautions should be observed in all stages of the manipulations; (3) the benzoic acid should be evaporated spontaneously—without aid of any heat other than that of room temperature—and drafts should be avoided.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the volumetric method be adopted as official.
- (2) That the gravimetric method be adopted as tentative.
- (3) That the amended A. D. M. A. method be adopted as tentative.
- (4) That no further work be done on this project.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 72.

REPORT ON CHAULMOOGRA OIL.

By L. E. WARREN (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

In 1924¹ considerable collaborative work was done on chaulmoogra oil. The tests given in New and Non-official Remedies, 1924, and which were proposed for the U. S. P. X, were tried on several specimens of the oil, but they were considered inadequate to prevent adulteration with some foreign oils, especially castor oil and croton oil. Tests were devised, depending on the greater solubility of these oils in alcohol, and determination was made of the iodine number and saponification number of the alcohol-soluble fraction, as well as of the viscosity of the original oil, which with the N. N. R. tests were considered sufficient to detect the most likely adulterations. Some discrepancies in the results obtained by the collaborators were noted.

In 1925² the inequalities reported in the previous year were for the most part adjusted. During the year the U. S. P. X became official, and this edition included tests for the purity and identity of chaulmoogra oil. The U. S. P. X methods for determining optical activity, iodine absorption number, saponification number, and acid number were made official. However, the alcohol solubility and the viscosity tests reported by the associate referee in the previous year were not included in the Pharmacopeia. Accordingly these tests were recommended to be adopted as tentative methods by the A. O. A. C.

In 1926³ no collaborative work was reported by the associate referee. A review of the literature was given and it was shown that chaulmoogra oil and its derivatives were being used increasingly in the treatment of leprosy and with good results. A new oil from Brazil (*Carpotroche brasiliensis*) was reported as being in successful use by the physicians of Brazil for the treatment of leprosy. The chemical and physical properties of this oil were examined by the French chemists, André and Moureu⁴, and the results were abstracted by the associate referee.

Since the last meeting of the A. O. A. C. specimens of this oil have been obtained by the Department of Agriculture and have been examined by G. S. Jamieson of the Oil, Fat and Wax Laboratory, Bureau of Chemistry and Soils, and his assistants. This year three color tests for chaulmoogra oil which were found in the literature were tried by the associate referee with a view to sending out samples of oil for collaborative work if the tests should prove sufficiently worth while. The tests are given herewith:

¹ *This Journal*, 1925, 8: 515.

² *Ibid.*, 1926, 9: 290.

³ *Ibid.*, 1927, 10: 349.

⁴ *Compt. rend.*, 1925, 181: 1089.

TABLE 1.
Color tests applied to oils of the chaulmoogra series and to some other oils.

KIND OF OIL	T. KUMZII (AUTHENTIC)	T. KUMZII (MARKET)	T. KUMZII (MARKET)	T. KUMZII (MARKET)	CAMPOTOCHE BHASILIENSIS	HYDNOCAEPUS WIGHTIANA	ONCOSA MCHINATA	RICINUS COMMUNIS	GOSSEYUM	ARACHIS HYPOGAEA	BRASSICA RAPUS
Test No. 1	Grass green	Dirty yellowish green	Grass green	Dirty grass green	Dirty yellowish green	Grass green	Grass green	Very faint pinkish	Dirty purple	Slight purple	Dirty brownish pink
Test No. 2	Dirty violet green	Reddish brown	Reddish brown	Dark brown	Reddish brown	Dirty green	Pale green	No color	Dirty purple	Pale violet	Dark bluish red
Test No. 3	Dirty violet	Dirty greenish violet	Dirty violet	Dirty greenish violet	Bluish violet	Dirty violet	Bluish violet	No color	No color	Light violet purple	Light violet purple

1. Dissolve 1 drop of chaulmoogra oil in 0.5 cc. of chloroform and dilute the mixture with 1.5 cc. of glacial acetic acid. Add 4 or 5 drops of sulfuric acid. A grass-green color, which is reddish violet by transmitted light, develops gradually. On standing the color is most pronounced in the drops of sulfuric acid that separate from the mixture¹.

2. Add 1 drop of sulfuric acid to 5 cc. of a chloroform solution (1 : 10) of the oil and shake; a beautiful green color is produced after a short time.

3. Add 5 drops of a mixture of 1 gram of trichloroacetic acid and 4 drops of hydrochloric acid to 10 drops of the oil and warm gently; a deep blue color should be produced².

The results, which are given in Table 1, were distinctly disappointing. The colors expected were not sharp, and in some cases those found varied considerably from the standards set. Consequently no collaborative work was sent out by the associate referee. It was found that the tests, while more or less distinctive for oils of the chaulmoogra group, were of no value in distinguishing the individual oils in the group. Moreover, the tests were applied to four commonly occurring oils (peanut, cottonseed, rape, and castor) and while the color reactions of the chaulmoogra group were not duplicated with these common oils, nevertheless in some cases distinct colors were produced, and in a few instances they might be confusing with reference to the chaulmoogra group.

RECOMMENDATIONS.

It is recommended—

(1) That no color reactions for the oils of the chaulmoogra group be adopted.

(2) That since chaulmoogra oil is described in the U. S. P. X, and certain other tests have been adopted by the A. O. A. C. as tentative, no further work on chaulmoogra oil be carried out at this time.

No report on crude drugs was given. The associate referee substituted the following report on fluidextract of ginger.

REPORT ON FLUIDEXTRACT OF GINGER.

By J. F. CLEVINGER (Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

No assay is given in United States Pharmacopeia X for the fluid-extract of ginger, and the assay given for the crude drug is not adequate to detect an adulterated extract. Therefore an investigation of ginger and its preparation was undertaken. As a result the following method of analysis of ginger is proposed.

¹ Lifschütz. *Chem. Ztg.*, 1921, 45: 1264.

² Jap. Pharm., 4th ed., p. 289.

METHOD FOR DETERMINATION OF FLUIDEXTRACT OF GINGER.

Place 50 cc. of fluidextract of ginger in a 100 cc. round-bottomed flask and evaporate the alcohol on the steam bath, using a current of air. (Care should be taken to avoid driving off any of the volatile oil from the residue.) Add 50 cc. of water to this residue and determine the quantity of volatile oil present, using a special apparatus devised for this purpose¹. Place the flask in an oil bath and boil approximately 2 hours. The number of cubic centimeters of oil obtained multiplied by 2 gives the percentage of volatile oil. Finally transfer the volatile oil to a test tube (10 x 75 mm.) and allow to stand until perfectly clear (overnight is usually sufficient). Determine specific gravity, the index of refraction, and the optical rotation of the oil thus obtained. The limited quantity of oil usually obtained (0.75-1.5 cc.) necessitates the determination of the optical rotation in a 50 mm. micropolarizing tube. The specific gravity may be determined in a Sprengel specific gravity bottle of approximately 0.5 cc. capacity.

Transfer the contents remaining in the flask to a separatory funnel and shake out with ether until the ether solution is practically free from color. (Three or four shake-outs with 50 cc. portions of ether is usually sufficient.) Transfer the ether portion thus obtained to a tared beaker and evaporate to dryness on a steam bath, using an air current. Place the beaker containing the residue in a vacuum desiccator containing sulfuric acid for approximately 2 hours and weigh. The ether-soluble solids so found are considered to be the active constituents of ginger. They will hereafter be designated as *G*. The weight in grams multiplied by 2 gives the percentage of *G*. Dissolve in neutralized 95 per cent alcohol and make up to a volume of 50 cc., using care to obtain a uniform solution. Take 10 cc. portions of this solution and determine the iodine and saponification values by the following modifications of the U. S. P. methods.

IODINE VALUE.

Transfer 10 cc. of the prepared solution into a glass-stoppered bottle of 250 cc. capacity and evaporate to dryness on the steam bath, using an air current. Dissolve in 10 cc. of chloroform. Add 15 cc. of iodobromide T. S. (U. S. P. X, p. 489) measured from a buret. Stopper the bottle securely and allow the mixture to stand for half an hour in a cool dark place. Shake the mixture at 10 minute intervals. Then add in the order named 20 cc. of potassium iodide T. S., 75 cc. of distilled water, and 0.1 *N* sodium thiosulfate in small successive portions, shaking thoroughly after each addition until the color of the mixture becomes quite pale. Now add a few drops of starch T. S. and continue the addition of 0.1 *N* sodium thiosulfate until the blue color is discharged.

Make a blank test by mixing exactly the same quantities of reagent and titrate the free iodine with 0.1 *N* sodium thiosulfate as directed above. The difference in the number of cubic centimeters of 0.1 *N* sodium thiosulfate consumed by the blank test and the actual test, multiplied by 1.269 and divided by the weight of *G* taken, gives the iodine value.

SAPONIFICATION VALUE.

Transfer 10 cc. of the *G* solution into an Erlenmeyer flask of approximately 200 cc. capacity and add 10 cc. of alcoholic half-normal potassium hydroxide (U. S. P. X, p. 501). Through a perforated stopper, insert into the neck of the flask a glass tube from 70 to 80 cm. in length and from 5 to 8 mm. in diameter, and heat the flask on a water bath for half an hour, frequently rotating the contents. Then add 1 cc. of phenolphthalein T. S. and titrate the excess of potassium hydroxide with half-normal hydrochloric acid.

Make a blank test, using exactly the same quantity of alcoholic half-normal potassium hydroxide. The difference in the number of cubic centimeters of half-normal hydrochloric acid consumed in the actual test and in the blank, multiplied by 28.06 and divided by the weight of *G* taken, gives the saponification value.

¹ *J. Am. Pharm. Assoc.*, 1928, 17: 345.

The natural color of ginger interferes with the determination of the true end point since the red color of the phenolphthalein grades off imperceptibly into the brown of *G*. The natural color of *G* varies considerably, depending primarily upon the variety of ginger used. Two distinct color changes occur during the titration—first when the red of the phenolphthalein disappears and second the change from dark to light brown. This latter change gives the true end point as determined by electrometric titration.

Samples of fluidextract of ginger were prepared by the U. S. P. method from African and Jamaican varieties of ginger and divided into two lots each. To one lot of each variety a definite quantity of castor oil was added. Sample No. 1 consisted of the fluidextract of African ginger, Sample No. 2 consisted of No. 1 with an addition of castor oil amounting to approximately 20 per cent of the total solids, Sample No. 3 consisted of the fluidextract of Jamaica ginger, and Sample No. 4 consisted of No. 3 with an addition of castor oil amounting to approximately 40 per cent of the total solids.

Subsamples were made of these four lots and were submitted, together with special apparatus and instructions, to the following collaborators: A. C. Blaisdell of the Bureau of Prohibition and D. M. Copley of the Norwich Pharmaceutical Company.

Collaborative results.

SAMPLE NO.	VOLATILE OIL			NON-VOLATILE ESTHER EXTRACT		
	<i>per cent</i>	Ref. Ind.	Op. Rot.	<i>per cent</i>	I. No.	Sapon. No.
Blaisdell						
1	2.7	1.496	6.8	37	100
2	3.0	1.498	7.2	35.9	78.3
3	0.35	1.662	3.5	62.1	124.0
4	0.7	1.656	5.7	48.4	121.8
Copley						
1	2.6	7.5	14.5	88.6
2	2.45	8.3	15.4	145.9
3	0.9	3.3	22.1	131.0
4	1.0	5.4	15.9	153.4
Clevenger						
1	2.9	1.496	−45°	6.4	34	58
2	2.9	1.496	−45°	8.0	37	65
3	1.2	1.490	−45.5°	2.96	45.5	72.3
4	1.2	1.490	−45.5°	5.1	57.3	120.7

Copley's comments are as follows:

The refractive index and optical rotation of the oil were not taken owing to lack of suitable apparatus. * * *

Du Mez in his work on oleoresins found oleoresin ginger to give quite an appreciable acid number. It is possible that this procedure should be applied to the gingerol (active constituent of ginger) and allowance made for the acid number in determining the saponification value. I have no confidence in the above iodine and saponification values due to the blind end points, the solutions being colored dark brown by the gingerol. An analyst thoroughly familiar with these titrations would probably be able

to determine the correct end point. The titration of each iodine value was extremely slow since after an apparent end point had been reached gentle agitation would free more uncombined iodine and cause the indicator to again turn blue. The titrations for the saponification number were equally uncertain. I reached the above conclusion by taking a similar portion of the gingerol solution at frequent intervals during the titration and determining its reaction by spot testing. Even this procedure was not very satisfactory. I question the value of these two determinations except when run by an analyst experienced in this particular work.

Copley's comments regarding "blind end point" for both the iodine and saponification values do not, in the experience of the associate referee, apply equally in the two determinations. The lack of a more detailed direction for the carrying out of this work as originally sent out to collaborators no doubt accounts for the unsatisfactory results obtained.

These methods are somewhat empirical and depend to some extent upon the experience of the analyst. The writer has found that the end point for the iodine value of the resin, especially of African ginger, is somewhat obscured if sufficient time is not allowed for particles of the resin to settle after shaking the flask. It must also be noted that iodine is always set free from the solution in small quantities after the end point has been reached in case of the titration of any fat or oil. This is due to a secondary reaction. The associate referee had no difficulty in obtaining a satisfactory end point for the determination of the iodine value.

The determination of the saponification value in the case of this resin presents greater difficulties than the iodine value owing to the interference of the brown color in the resin. However, a little experience should enable the analyst to obtain good results. The end point in this instance was determined electrometrically, and as it has been pointed out previously it is not where the red disappears in the titration, but where the dark brown color changes rather abruptly to light brown. It is also important that the volatile matter be entirely removed from the resin before determining the iodine and saponification values. Otherwise the results obtained will be too high.

It is recommended that this work be continued during the coming year¹.

REPORT ON CHLOROFORM AND CARBON TETRACHLORIDE.

By W. F. KUNKE² (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

The time of the associate referee available for this work was devoted to preliminary experimental study. No collaborative work was sent out.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 74.

² Presented by V. K. Chesnut.

The first problem was in connection with a satisfactory procedure for obtaining accurately the weight of chloroform used in preparing samples. Obviously such samples are essential in the study of the various methods for the quantitative estimation of chloroform. It was thought that an improvement in the procedure for the preparation of collaborative samples given in former A. O. A. C. reports¹ could be worked out.

There is, no doubt, an unavoidable loss due to evaporation in transferring a 100 gram sample of chloroform, accurately weighed in a 100 cc. volumetric flask, to a liter graduated flask. To avoid this loss of chloroform, it was decided to take under-water weighings of the chloroform drained from a certain pipet.

The 65 cc. Erlenmeyer-shaped flask in which the chloroform weighings were made had a ground-glass stopper. The stopper opening was 2.5 cm. in diameter, and the flask without the stopper was 7.5 cm. high. The stoppered flask, containing about 15 cc. of water, was weighed. It was found that 30 seconds is a convenient period of time in which to remove the glass stopper, drain the chloroform from the 5 cc. pipet used, and replace the stopper. During this time the loss in water due to evaporation was negligible, varying according to the weighings taken from nothing to 0.5 mg. The pipet was held just above the top level of the water in the flask, and as the level of the water was raised by the draining of the chloroform into the flask, the pipet was correspondingly raised so as to avoid contact with the water. In each case the period between removing and replacing the stopper was 30 seconds. After the stopper was replaced the flask containing the water and chloroform was weighed. The difference in weight was the weight of the chloroform. The average of these weights, made under the same conditions, was assumed to be the weight of the chloroform drained from the pipet. The following results were obtained:

LOSS IN WATER, FLASK OPEN 30 SECONDS	WEIGHT OF CHLOROFORM
<i>mg.</i>	<i>gram</i>
0.2	7.4180
0.0	7.4255
0.3	7.4063
0.0	7.4165
0.0	7.4225
0.5	7.4155
0.0	7.4077
	7.4156 (Average)

The maximum variation in weight of the chloroform from the average weight was 0.0103 gram, or 0.139 per cent. The chloroform drained from the same pipet under the same conditions may be used to make a sample of known chloroform content, which is reasonably accurate for

¹ *This Journal*, 1926, 9: 297.

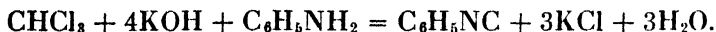
quantitative work. The chloroform is drained directly from the pipet into the volumetric flask used to make the sample. The flask should contain enough water to cover the chloroform entirely, and care should be taken to keep the temperature the same as that in the original weighings and to hold the pipet just above the water level. It should not touch the water. No weighing is made. The weight of the chloroform used is assumed to be the average of the weights found in the original weighings.

The second problem was to determine the influence of various reagents together with alcoholic potassium hydroxide upon the quantity of chloride formed from chloroform with the hope that a simple quantitative method could be worked out without the use of the pressure flask. The pressure flask was not used in any of this work. Experiments were made with or without the use of heat, and the reaction period was varied from 30 minutes to 48 hours.

The chloroform sample used—a solution of chloroform in water—contained 0.292 gram of chloroform per 100 cc. In each determination the 25 cc. aliquot (equivalent to 0.073 gram of chloroform) was drained from a 25 cc. pipet into a 300 cc. Erlenmeyer flask which contained 25 cc. of alcoholic potassium hydroxide. This reagent was made by dissolving 30 grams of potassium hydroxide in 30 cc. of water, cooling, and diluting to 100 cc. with methyl alcohol. Then 25 cc. of alcohol (95 per cent) was added except where noted in Table 1. Anilin, Devarda's alloy, or aluminum was used, respectively, as indicated. Devarda's alloy was a No. 20 mesh or finer, and the aluminum wire (No. 16) weighed about 0.1 gram per inch. The flask was loosely stoppered or covered with a watch glass, except where noted. After evaporating most of the alcohol, the chloride formed was determined gravimetrically as silver chloride.

The results indicate that aluminum or Devarda's alloy is useless, owing, perhaps, to the liberated hydrogen, which carries along chloroform and is lost to the determination. As the table shows, the results are lower than when only alcoholic potassium hydroxide is used. It was hoped that the nascent hydrogen would accelerate the rate of formation of the chloride from the chloroform, not only to shorten the reaction period but also to obtain a quantitative amount of chloride.

Anilin was used because it was thought that it might promote a rapid quantitative formation of chloride. The isonitrile reaction takes place as follows:



Although phenyl carbylamine or isonitrile has an intensely nauseous odor and on first thought might be considered disagreeable to work with, no odor is noticed after making the liquid acid, in preparation for the precipitation of the chloride formed as silver chloride, because isonitriles

TABLE 1.
Results obtained by associate referees.

EXPERIMENT NO.	TEMPERATURE	REACTION PERIOD	REAGENT ADDED	SILVER CHLORIDE FOUND	CHLOROFORM FOUND (ON BASIS OF 0.073 GRAM/25 cc.)
1	Room	18 hours	gram (a)	per cent 54.8
2	"	" "	Devarda's Alloy (1 gram)	0.0843	32.1
3	"	" "	Aluminum (0.2 gram)	0.0876	33.3
4	"	" "	Aluminum (0.3 gram)	(b)	43.3
5	"	" "	Aluminum (0.2 gram) Devarda's Alloy (0.5 gram)	0.0704	26.9
6*	88°C.	45 minutes	1 cc. Anilin	0.1810	68.8
7*	"	1 hour	" "	0.1779	67.7
8*	"	2 hours	" "	0.1810	68.8
9	Room	1½ "	" "	0.2070	78.7
10	"	24 "	" "	0.2268	86.3
11	"	48 "	" "	0.2250	85.6
12	"	48 "	" "	0.2245	85.4
13†	Steam bath	30 minutes	" "	0.2180	82.9
14†	" "	15 "	" "	0.2170	82.5
	Room	40 "	" "		
15†	Steam bath	15 "	" "	0.2061	77.1
	Boiled	10 "			
	Room	75 "			

(a) 10.3 cc. 0.1 N AgNO₃.(b) 13.65 cc. 0.1 N AgNO₃.

* No 95 per cent alcohol was added.

† Flask was stoppered with a one-hole rubber stopper with a 3/8 inch glass tubing 2 feet long inserted.

are readily decomposed by dilute mineral acids. It is only after the solution used in the determination has been made acid that it is exposed to the air. In short, the use of the isonitrile reaction does not make the procedure disagreeable.

CONCLUSION.

Weighing the chloroform under water is a simple, satisfactory, and accurate procedure for determining the weight of chloroform drained from a pipet.

Devarda's alloy or aluminum in an alcoholic potassium hydroxide solution does not appear to promote a higher yield of chloride, other conditions being the same.

The use of the isonitrile reaction appears to give a higher yield of chloride than the use of alcoholic potassium hydroxide alone.

RECOMMENDATION¹.

It is recommended that further study be made of the isonitrile reaction with the aid of a reflux condenser apparatus in the quantitative determination of chloroform.

REPORT ON IPECAC ALKALOIDS.

By A. R. BLISS, JR. (College of Medicine, University of Tennessee, Memphis, Tenn.), *Associate Referee*.

Following the recommendations made at the last meeting, the collaborators and the associate referee devoted their time to: (1) the Palkin-Watkins hand extraction method; (2) the Palkin, Murray and Watkins automatic extractor method; (3) the Palkin-Watkins purification method; and (4) the U. S. P. X assay.

METHODS.

FLUIDEXTRACT OF IPECAC.

METHOD I. Palkin-Watkins Hand Extraction Method.

This method has been published².

METHOD II. Palkin, Murray and Watkins Automatic Extraction Method.

This method has been published³.

METHOD III. Palkin-Watkins Purification Method.

This method has been published⁴.

METHOD IV. U. S. P. X Assay.

See U. S. P. X, pages 170, 200, 453.

COLLABORATIVE WORK.

The sample of fluidextract of ipecac used in the studies was a manufacturer's product. The collaborators' reports are given in the table.

Reports were received from the following collaborators:

H. R. Watkins, U. S. Food, Drug and Insecticide Administration, Washington, D. C.
S. Palkin, U. S. Food, Drug and Insecticide Administration, Washington, D. C.
H. O. Moraw, Chemical Laboratory, Swan-Myers Company, Indianapolis, Ind.
F. C. Synkovich, U. S. Food, Drug and Insecticide Administration, Chicago, Ill.
M. F. Brown, University of Tennessee, Memphis, Tenn.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 73.

² *J. Am. Pharm. Assoc.*, 1924, 13: 694; *This Journal*, 1928, 11: 50.

³ *Ind. Eng. Chem.*, 1925, 17: 612; *This Journal*, 1928, 11: 50.

⁴ *This Journal*, 1927, 10: 361.

The collaborators commented as follows:

H. R. Watkins.—While these results appear to be in excellent agreement, it may be stated that very erratic results were obtained at first. These on close investigation were traced to impure ether used in the extraction. While full information is not as yet at hand regarding all the impurities in bad ether which affect the ipecac alkaloids, it was found that peroxides had notably destructive effect. Ether specially purified in the laboratory and a commercial brand of ether, which was found free from peroxide, were used in the reported determination. Further work is contemplated on this subject, and the results of investigation will probably be published in the near future.

H. O. Moraw.—I obtained two other results by the mechanical extractor method, namely, 1.87 grams per 100 cc. and 1.73 grams per 100 cc., but do not wish to report them with the other results by this method for the reason that I doubt if complete extraction was obtained. The same indefiniteness regarding the exact procedure with the mechanical extractor method as existed last year necessitated experimenting with the amount of ammonia used to neutralize the acid. It is believed that more uniform results could be obtained if the exact procedure could be secured from the authors and sent to the collaborators.

F. C. Synkovich.—The hand extraction method is rapid and with gentle shaking no emulsions were encountered. Shaking with ether eight times completed the extraction.

The U. S. P. X method is long and tedious. Even with gentle shaking the extraction with ether forms emulsions which are difficult to break up.

The mechanical extractor carried over a small amount of water into the ether extract, giving it a yellow color. The end point in the titration was indistinct. By separation of the water in a separatory funnel the end point is satisfactory.

M. F. Brown.—The U. S. P. X assay is practically useless owing to the emulsions that are formed.

TABLE 1.
Collaborative results.

ANALYST	U. S. P. X	PURIFICATION METHOD	HAND EXTRACTION	AUTOMATIC EXTRACTION
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
H. R. Watkins	Impossible Emulsions	2.34 2.33	2.36 2.37	2.42 2.42
S. Palkin	Impossible Emulsions	2.26	2.33	2.4 2.35
H. O. Moraw	. . .	2.30*	2.21	2.30
F. C. Synkovich	2.04	. . .	2.28 2.25	2.12 2.17
M. F. Brown	Impossible Emulsions	2.24 2.22	2.30 2.26	2.34 2.34
A. R. Bliss	Impossible Emulsions	2.20 2.24	2.32 2.34	2.38 2.30

* Alcohol used to break emulsions on first extractions and ether only on third extraction.

CONCLUSIONS.

The U. S. P. X assay is unsatisfactory.

The Palkin, Murray and Watkins automatic extraction method appears most satisfactory.

The Palkin-Watkins hand extraction method is more rapid than and yields results almost as high as the automatic extraction method. (Ether free from peroxides must be used in the extraction.)

RECOMMENDATION¹.

It is recommended that the Palkin, Murray and Watkins automatic extraction method and the Palkin-Watkins hand extraction method be adopted as tentative.

H. R. Watkins: Our laboratory became interested in the assay of ipecac about three years ago through the development of the automatic extractors. We found that we could obtain almost twice as much ether-soluble alkaloids in some cases by means of the acid purification and automatic extraction as was obtained by the method of assay prescribed in U. S. P. IX. An interesting incident occurred in connection with one of the official samples this year. We had obtained certain results, which we were about to send to Dr. Bliss. I was making an assay by the U. S. P. X method, which required the use of large volumes of ether. When it came to making the titration I found no alkaloid at all. However, I went through all the operations again, and this time just as I passed the steam bath a slight explosion occurred. The ether was known to contain peroxide, and it appears that this was the cause of the explosion. It happened that we had a certain brand of ether which had been found to be absolutely free from peroxide, every unit having been tested separately. I used that supply to obtain the results which are recorded in Dr. Bliss's report. This work led us to think that the presence of peroxide in ether does affect the alkaloids of ipecac, and we contemplate studying them, more especially in regard to the effect of the presence of peroxide.

L. E. Warren: I was one of the collaborators on cocaine. After Mr. Watkins had discovered that ether containing peroxide influenced the results on ipecac alkaloids, I also tried the method on cocaine, using ether which was known to contain peroxide. I found there that it also influenced the results, so that they were practically worthless in the determination of cocaine. I didn't mention that fact in making my report to Mr. Eaton, who is the Associate Referee on Cocaine. I ran through the determinations twice and got no results that were worth recording, but I could get good results with ether known to be free from peroxide.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 73.

REPORT ON RADIOACTIVITY IN DRUGS AND WATER.

By J. W. SALE (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

With a view to carrying out last year's recommendation, that a collaborative study be made of the procedures for the preparation for analysis of solid, semi-solid, and liquid samples, a series of synthetic samples containing known quantities of radium were prepared as follows:

A. Samples completely soluble in acids:

No. 1 Solid: Table salt dissolved in water mixed with a definite quantity of standard radium solution, evaporated to dryness. Residue pulverized and mixed.

No. 2 Clear liquid: Washington city tap water mixed with a definite quantity of standard radium solution.

B. Samples insoluble or incompletely soluble in acids:

No. 3 Solid: C. P. silicic acid in powder form added to standard radium solution, evaporated to dryness, and mixed.

No. 4 Semi-solid: Incompletely soluble in acids. Mixture of glucose, cocoa, and standard radium solution.

No. 5 Liquid immiscible with water. Emulsion of corn oil, acacia, and standard radium solution.

No. 6 Liquid containing material which is insoluble in dilute nitric acid. A suspension of kaolin in standard radium solution.

Next year these samples will be submitted to collaborators, who will prepare them for analysis and determine the content of radium according to the procedures described in the reports of 1924 and 1926¹.

RECOMMENDATIONS.

It is recommended—

(1) That the synthetic samples which have been prepared be submitted to collaborators for determination of radium content according to procedures recommended in the reports of 1924 and 1926 on radioactivity in drugs and water.

(2) That the associate referee prepare a description of the preparation of a standard stock solution of radium.

No report on laxatives and bitter tonics was given by the associate referee.

¹ *This Journal*, 1925, 8: 531; 1927, 10: 362.

REPORT ON MERCURIALS.

By ROBERT S. ROE¹ (U. S. Food, Drug, and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

In accordance with the recommendation of last year, further study was made of the method proposed for the assay of calomel in tablets as well as of other methods, in an effort to shorten the procedure.

The following basic methods were considered by the associate referee: (I) Sulfide method as reported by P. W. Morgan last year²; (II) the Jamieson iodate method³; (III) the Jamieson thiocyanate method⁴; and (IV) a modification of the U. S. P. iodine method for calomel. This last method is essentially as recommended by the Contact Committee.

(I) *The Sulfide Method.*

The sulfide method is based on the work of A. W. Bender⁵ on mercuric iodide tablets. The sample is treated with 1 : 1 hydrochloric acid and potassium chlorate to oxidize and dissolve the calomel as mercuric chloride, and to destroy the organic excipients. After filtering and nearly neutralizing with ammonia, the mercury is precipitated and weighed as mercuric sulfide.

While in general good results were obtained, it is difficult to filter the precipitate without loss, and unless the acidity is carefully controlled the precipitate is not coagulated properly. Owing to the many objections to the use of the sulfide method, it was thought best to give preference to other methods that are equal in accuracy and perhaps more satisfactory in manipulation.

(II) *The Iodate Method.*

The iodate method consists in the titration of the calomel with potassium iodate in the presence of hydrochloric acid, chloroform being used as the indicator. High results are obtained by this method when applied direct to tablets, because in the presence of hydrochloric acid excipients such as lactose reduce the calomel to metallic mercury.

It was thought that this difficulty might be avoided by dissolving and removing the lactose, by filtration, and then placing the filter paper containing the insoluble residue in the titration flask. Fairly good results were obtained, but starch and other insoluble materials tend to emulsify the mixture, masking the indicator. If the starch is hydrolyzed by warming the acid mixture, some calomel is reduced and results are high. This method, therefore, was considered unsuitable.

¹ Presented by C. F. Pappé.

² *This Journal*, 1927, 10: 367.

³ *Volumetric Iodate Methods*, p. 51.

⁴ *J. Ind. Eng. Chem.*, 1919, 11: 296.

⁵ *Ibid.*, 1914, 6: 753.

(III) The Thiocyanate Method.

The thiocyanate method of Jamieson was suggested by Spencer for the assay of mercuric chloride in antiseptic tablets¹. This method employs the formation of a precipitate of mercury zinc thiocyanate in slightly acid solutions of mercuric chloride by the addition of a solution of zinc ammonium thiocyanate. The precipitate is filtered and either weighed or titrated with potassium iodate.

In applying this procedure to calomel, it is necessary first to oxidize the calomel to mercuric chloride, as is the case in the sulfide method. The results obtained are well within the limits of accuracy required for this work.

Although the method step by step parallels the sulfide procedure, it is more rapid in that the precipitation is clean-cut and the filtration is much more rapid and satisfactory.

(IV) The Iodine Method.

The inapplicability of the U. S. P. iodometric assay for calomel to the assay for calomel in tablets is due largely to the presence of lactose and other soluble reducing excipients.

The modified method proposes the treatment of the sample with water to remove the soluble fillers. The undissolved residue containing the calomel is then treated with potassium iodide and an excess of standard iodine as in the U. S. P. procedure.

The end point of the titration of the excess iodine with thiosulfate is not sharp, owing to the presence of starch granules and filter paper which absorb and retain iodine. However, by running in an excess of the thiosulfate and titrating back with iodine after all the color has disappeared from the paper, a satisfactory end point is obtained.

The method as outlined by the Contact Committee fails to provide sufficient time for the oxidation of the calomel in the iodine treatment. Hence, the time of this procedure is increased from 30 to 90 minutes. It is necessary to agitate the flask frequently during this period to break up the filter paper so that the iodine will have free access to the calomel contained therein. The results obtained by this method are also within the required limits of accuracy.

Typical results obtained by the associate referee by the four methods considered are shown in Table 1.

METHODS FOR COLLABORATIVE STUDY.

Method III, the thiocyanate method, including both gravimetric and volumetric forms, and Method IV, the modified iodine method, were selected for collaborative study.

¹ *This Journal*, 1925, 8: 538.

TABLE 1.
Results obtained by associate referee, using four methods described.

SAMPLE	HgCl CALCULATED	HgCl FOUND		RECOVERY	METHOD USED	REMARKS
		gram	per cent			
1.5 grams Mixture A*	per cent 10	0.1512	10.08	per cent 100.80	Sulfide	Titrated direct—no preliminary treatment
2 grams Mixture A*	10	0.2039	10.19	101.90	Sulfide	
0.2 gram HgCl	100	0.2039	101.95	101.95	Iodate	
0.2 gram HgCl 0.4 gram Starch 0.4 gram Lactose	20	0.2159	21.59	107.95	Iodate	
0.1 gram HgCl 0.5 gram Starch 0.4 gram Lactose	10	0.1060	10.60	106.00	Iodate	Lactose dissolved in water and filtered off. Paper returned to flask. Starch hydrolyzed before titrating.
0.1 gram HgCl 0.5 gram Starch 0.4 gram Lactose	10	0.10053	10.05	100.53	Thiocyanate (gravimetric)	
1.5 grams Mixture A*	10	0.1535	10.23	102.30	Thiocyanate (gravimetric)	
2 grams Mixture A*	10	0.1990	9.95	99.50	Thiocyanate (volumetric)	
0.15 gram HgCl 0.60 gram Lactose 0.25 gram Starch	15	0.1494	14.94	99.60	Iodine	

* Mixture A— 5 grams HgCl.....10%
20 grams Starch.....40%
25 grams Lactose.....50%

Two samples were prepared for the collaborative work. Sample No. 1 was prepared to contain 11.50 per cent of calomel, 15 per cent of starch, 50 per cent of lactose, and 23.50 per cent of powdered sucrose. Sample No. 2 was a portion of the calomel used in sample No. 1.

DIRECTIONS FOR COLLABORATORS.

The following directions were sent to the various collaborators:

CALOMEL TABLETS.

Method III—Thiocyanate Method.

REAGENTS.

- (a) *Hydrochloric acid.*—1 : 1.
- (b) *Thiocyanate-zinc sulfate solution.*—Dissolve 39 grams of ammonium thiocyanate and 50 grams of zinc sulfate in water and dilute to 1 liter.
- (c) *Dilute thiocyanate-zinc sulfate (wash solution).*—Dilute 10 cc. of (b) to 500 cc. with water.
- (d) *Standard iodate solution.*—Dissolve 27.1992 grams of pure, dry potassium iodate in water and dilute to 1 liter. One cubic centimeter of this solution is equivalent to 0.005 gram of calomel. The potassium iodate should be powdered and dried at 120°C. before using.

PROCEDURE.

Count and weigh a representative number of tablets to ascertain the average weight. Pulverize the sample and weigh a portion of the well-mixed powder representing 3-4 grains of calomel. Transfer to a 250 cc. Erlenmeyer flask. Add 10 cc. of 1 : 1 hydrochloric acid, rotating the flask to prevent lumping of the sample. (If necessary use more than 10 cc. of the 1 : 1 hydrochloric acid. The final acidity of the filtered solution at the time of the addition of the thiocyanate reagent should not exceed 5 per cent. If necessary, the acidity may be reduced with sodium bicarbonate.) Add about 0.5 gram of potassium chlorate, cover the flask with a small watch glass, and allow the mixture to digest at room temperature for about 30 minutes. Place the flask on a steam bath and digest for 15-20 minutes longer. Dilute the solution to about 40 cc. Fit a two-hole rubber stopper to the flask and through one hole insert a glass tube extending below the surface of the solution; into the other hole insert a short tube and connect it with a water suction pump. Aspirate to remove all chlorine by drawing air through the solution. Twenty minutes is ordinarily ample time for this operation. Filter into a 400 cc. beaker and wash well with water until the volume is about 125-150 cc.

Add 30 cc. of the thiocyanate-zinc sulfate reagent. Hasten the separation of the crystals by striking the outside of the beaker with a glass rod. When the precipitate has formed, stir well with a glass rod. Then allow the precipitate to settle for about an hour before filtering.

(a) *Gravimetric Estimation.*

Decant the above precipitate through a weighed Gooch crucible. Transfer the precipitate to the crucible and wash 4 or 5 times with the dilute thiocyanate-zinc sulfate solution. Finally wash with 10 cc. of water. Dry for an hour or to constant weight at 100°-110°C. and weigh.

The weight of the precipitate multiplied by 0.47376 represents the weight of calomel in the sample.

Results obtained by collaborators.

CHEMIST	SAMPLE NO. 1—PER CENT CALOMEL PREPARED TO CONTAIN 11.5% CALOMEL				SAMPLE NO. 2—PER CENT CALOMEL (PURE CALOMEL USED IN SAMPLE NO. 1)			
	Method III (a)	Method III (b)	Method IV	Method Sulfide	Method U. S. P.	Method III (a)	Method III (b)	Method IV
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
A. W. Hanson	11.23 11.45 11.20 11.01	11.45 11.30	11.57 11.35			99.00 99.05 98.40 98.07	98.70 97.30	99.00 98.05
H. Wales	11.18 11.20	11.05 10.95	11.29 11.15			98.46 98.01	99.75 99.12	97.98 98.34
W. F. Kunke	11.12 11.10 11.14	11.20 10.90 10.85	11.26 11.35 11.50			99.40 99.20 99.40 99.10	98.00 99.25	100.03 99.70
E. L. Anderson	11.19 (a)* 11.31 (a) 10.83 (b) 10.75 (b)	11.20 (a) 11.40 (a) 10.90 (b) 10.88 (b)	11.50 11.50 11.45 (e) 11.19 (e)			98.45 (a) 98.45 (b)	101.50 (a) 101.50 (b)	99.87 99.87 99.16 (e) 99.16 (e)
D. L. Cottle	(c)	(c)	11.55 11.59			99.54 99.02	116.75 (d) 111.25 (d) 103.50	99.63 99.63
F. C. Synkovich	10.91 11.03	11.15	11.14 11.26			99.14 99.50 99.05	109.00 99.71 100.32	99.71 99.94 100.32
R. S. Roe	11.61 11.45 11.37 11.39 11.41	11.50 11.40 11.35 11.18	11.65 11.69 11.72 11.69	11.63 11.57 11.06 11.29	99.72 99.72 99.72	99.30 98.50	100.75 100.13	99.80† 99.55 100.90

* (a) The mixture turned brown on steam bath and remained brown after chlorine was removed. Precipitate was dark colored.

(b) Sample digested at room temperature without heating on steam bath. After removal of chlorine and addition of thiocyanate reagent, the mixture was allowed to stand overnight. Sample No. 1 precipitate turned brown, and odor of hydrogen sulfide was noted.

(c) Sample No. 1 precipitate came down yellow and after standing turned black with odor of hydrogen sulfide. This was not due to excess of potassium chlorate or incomplete removal of chlorine inasmuch as two determinations of sample No. 2, dechlorinated at the same time, did not show these results.

(d) Precipitation allowed to take place overnight, filtered on Gooch crucible, and dried at 110°C.

(e) Direct titration with iodine, disappearance of gray color being used as end point.

† Average of four determinations.

(b) *Volumetric Estimation.*

Filter the precipitate of mercury zinc thiocyanate obtained above through a small size filter paper supported by a platinum cone, using light suction. (The use of a Büchner funnel was also found to be satisfactory.) Wash four or five times with the dilute thiocyanate solution and transfer the precipitate to the paper. Finally wash once with 10 cc. of water.

Place the paper and precipitate into a 200 cc. glass-stoppered flask and add a cooled mixture of 20 cc. of water, 30 cc. of hydrochloric acid, and 6 cc. of chloroform. Shake and immediately titrate with the standard potassium iodate solution, adding the solution rapidly while rotating the flask. When the iodine that is liberated during the first stage of the reaction has disappeared from the solution insert the stopper and shake vigorously for about 30 seconds. Continue the titration slowly, shaking thoroughly after each addition until the iodine color just disappears from the chloroform indicator, which marks the end point.

The number of cubic centimeters of the iodate solution required times 0.005 represents the grams of calomel present in the sample taken for analysis.

NOTE: The mercury zinc thiocyanate is slightly soluble in pure water, hence the use of the dilute thiocyanate solution for washing the precipitate.

Method IV—Iodine Method.

This method has been published¹.

The results reported by the various collaborators are listed in Table 2.

COMMENTS BY COLLABORATORS.

A. W. Hanson.—The gravimetric method [III (a)] gives good results.

The volumetric method [III (b)], in which potassium iodate was used did not give as concordant results as the gravimetric method. The end point is not satisfactory in this titration.

The filter paper interferes somewhat with the titration of calomel by the iodine method (IV). The method as given calls for a large excess of the standard iodine solution. Any error in standardization of the solution will cause a considerable error in the results. The A. O. A. C. method for the preparation of standard iodine solution specified in this method takes a lot of time, and it is rather wasteful and messy. Some of the other methods for the preparation of the standard iodine solution would be preferable.

H. Wales.—The volumetric method [III (b)] is really a determination of thiocyanate instead of mercury. If the sample is not washed free of zinc ammonium thiocyanate, the results will be too high. It was first tried on sample No. 2 and approximately 102 per cent was found.

Method IV, the contact committee method, does not work on all samples. It is believed better to use the modification devised by Pappe², which is to hydrolyze the starch with 1-10 hydrochloric acid before filtering.

W. F. Kunke.—As a precaution it might be well to state in the thiocyanate method that the samples, after the precipitant has been added, should not be allowed to stand much longer than the prescribed time because of the reducing action of the lactose on the mercury compound.

D. M. Walsh (reporting the results of Anderson and Cottle).—We have used the potassium chlorate oxidation method on other mercurials, but have not found this a satisfactory method of preparation for the thiocyanate precipitation. We do not find that this oxidation interferes with the Rupp formaldehyde method.

¹ *This Journal*, 1928, 11: 61.

² Unpublished.

Most calomel tablets can be evaluated by the direct U. S. P. assay, with a preliminary washing with water. In cases where the end point is bad, we have been able to obviate this by a preliminary digestion with 1-9 hydrochloric acid and subsequent filtration and washing, thereby hydrolyzing and removing the starch which apparently causes the difficulty in the end point. Our preliminary work on this modification indicates a very slight loss of calomel, either through solution or oxidation, but the results obtained appear to be much more satisfactory than when the U. S. P. procedure is used. It seems probable that the addition of excess of thiosulfate and back titration with iodine, as outlined in your method No. IV, may solve this difficulty in a more satisfactory fashion.

We also wish to call your attention to the fact that contrary to the literature potassium iodate, even when practically dry, tends to deteriorate, and we have found it advisable to standardize solutions of potassium iodate against standard thiosulfate in the presence of excess of hydrochloric acid and potassium iodide.

F. C. Synkovich.—Sample No. 1, when digested on the steam bath with potassium chlorate and hydrochloric acid, turned dark if left on too long. If allowed to stand several hours, the precipitate obtained with the thiocyanate becomes dark. This is probably due to the organic matter present as no change in color was noted under the same conditions with sample No. 2.

The iodine method is the shortest of the three. The volumetric thiocyanate method is a little quicker than the gravimetric, inasmuch as the methods are the same up to the estimation of the precipitate and the titration can be made immediately in the former method, whereas in the latter it takes several hours to dry the precipitate to constant weight.

To test the methods further, a number of commercial tablets of calomel were assayed in accordance with the directions outlined for the collaborators. These results appear in Table 3.

TABLE 3.

SAMPLE	CALOMEL GRAINS PER TABLET DECLARED	CALOMEL—GRAINS PER TABLET FOUND				REMARKS
		Method III (a)	Method III (b)	Method IV	Method Sulfide	
A	1/2	0.484	0.483	
B	1/10	0.110	0.111	0.110	
B	1/10	0.114	0.111	
C	1	0.932	0.935	
D	1/2	0.472	0.468	Calomel and soda
E	1	0.917	0.910	
F	2	1.896	1.917	Calomel and soda
G	1	0.994	0.998	
H	1/2	0.496	0.505	

DISCUSSION.

It will be noted that the collaborators' reports indicate that the results obtained by method IV are very satisfactory. Those by method III (a) are also satisfactory on the whole, but method III (b) failed to give consistent results with all collaborators.

In regard to method III it might be well to recall that in attempting to utilize the thiocyanate reaction for the determination of mercuric chloride in tablets, Spencer found that the lactose present rendered the procedure unsuitable for the purpose¹. In applying this procedure to calomel, it was thought that the organic excipients would be sufficiently destroyed during the course of the oxidation to obviate this difficulty. Apparently there are oxidation products in solution which cause some decomposition if allowed to remain in contact with the mercury zinc thiocyanate precipitate longer than the time specified in the procedure. When the method is followed closely in detail as outlined, satisfactory results have been consistently obtained.

The work of the collaborators seems to indicate that method IV may be expected to give somewhat more dependable and consistent results. It is shorter than method III and requires only reagents and standard solutions that are commonly employed in many other determinations.

RECOMMENDATION².

It is therefore recommended that method IV be adopted as the tentative method for the assay of calomel in calomel tablets.

REPORT ON PYRAMIDON.

By FRED L. ELLIOTT (Food, Drug and Insecticide Administration),
Baltimore, Md.), *Associate Referee*.

On the recommendation of the Referee on Drugs that the proposed methods for pyramidon be further studied, two samples were submitted to various collaborators with a request that they be examined by each of the following methods (copies of methods submitted with samples):

Method 1.—Chloroform extraction from dilute alkali solution (A. O. A. C., Vol. 8, No. 5, p. 546).

Method 2.—Hydrochloride method (A. O. A. C., Vol. 8, No. 5, p. 547).

Method 3.—Chloroform extraction from ammoniacal solution (A. O. A. C., Vol. 9, No. 3, p. 309).

The results of the collaborators are given in the table.

¹ *This Journal*, 1926, 9: 307.

² For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 73.

TABLE 1.
Collaborative results.

COLLABORATOR	SAMPLE NO. 1 (U. S. P. PYRAMIDON)			SAMPLE NO. 2 MILK SUGAR—STARCH AND PYRAMIDON		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
J. A. Callaway, Jr. Food, Drug and Insec- ticide Admin. Savannah, Ga.	97.5 98.0	98.9 98.7	100.0 99.0	38.3 38.7	38.0 39.3	40.5 40.3
P. Cox Lehn & Fink Research Laboratories Bloomfield, N. J.	103.4† 104.1 98.36¶ 98.22	98.39 99.28	100.33 100.15	41.19 40.22	37.28 39.44	41.61 40.90
E. R. Miller Food, Drug and Insec- ticide Admin. New York, N. Y.	97.5	101.15† (Impurities)	99.95	38.0	39.85
L. G. Petree Food, Drug and Insec- ticide Admin. San Francisco, Calif.	99.30 98.70 99.30	95.70** 96.10	99.10 99.80	39.93 40.15	38.45 39.58	40.30 40.40
L. Freedman H. A. Metz Labora- tories, Inc. New York, N. Y.	97.60 97.95 98.20	104.60† 105.46† 105.21†	97.95 99.60 99.20	40.90 40.53 40.30	38.37 38.26 37.86	42.80 41.10 42.50
E. L. Anderson Food, Drug and Insec- ticide Admin. Baltimore, Md.	98.95 99.10	100.76* 100.98*	99.25 98.70	39.35 39.38	39.58 39.65	39.48 39.75
A. W. Hanson Food, Drug and Insec- ticide Admin. Minneapolis, Minn.	99.5 99.7	102.3* 101.7* 99.0†	99.4 99.5	40.0 40.5	40.9 40.4	40.38 40.66
W. F. Reindollar State Board of Health Baltimore, Md.	99.10†† 100.58†† 99.40 98.77	99.10* 99.57* 99.70† 99.90†	99.45 99.25	42.05†† 44.92†† 40.03 41.12	39.77 41.02	39.91 40.11
F. L. Elliott Food, Drug and Insec- ticide Admin. Baltimore, Md.	99.20 99.25	102.27* 102.06*	100.10 99.40	39.93 39.95	41.03 41.64	40.14 40.06
H. Wales Food, Drug and Insec- ticide Admin. Washington, D. C.	100.05 100.34	98.55* 98.50*	99.95 99.70	39.55 39.78	39.32 39.84	39.62 39.34
J. F. Ellis 2201 New York Ave., N. W., Washington, D. C.	100.00 100.49	100.16† 102.95† 100.00†	100.20 100.20	40.19 40.09	40.48 40.48	40.25 40.00
Average Theoretical	98.93	100.42	99.55	39.91 40.0	39.55 40.0	40.45 40.0
U. S. P.	(loss on drying 5 min. at 100°C.— 0.4 %)					

* Extracted residue used.

† Sample used without extraction.

‡ Stopper grease dissolved (not included in average).

¶ Pyramidon seen volatilizing at 98°C.

** Recovery from extraction residue: Sample No. 1—96.37% and 97.37%; Sample No. 2—96.30% and 97.95%.

†† Stopcock grease dissolved (not included in average).

COMMENTS OF COLLABORATORS.

J. A. Callaway.—The details of the extraction methods are complete and should insure a good yield, but neither is selective for pyramidon. Method 2, unless restricted to residue, is valueless.

P. Cox.—Qualitative tests—positive. Six extractions sufficient in Method 1.

L. G. Petree.—Method 2 gives erratic results. Since recovery of extraction is incomplete, a double loss occurs. Method 3 is preferred as a working method.

L. Freedman.—Methods 1 and 3 are satisfactory. Method 2—results unsatisfactory, drying difficult. Method 1 is simplest and most satisfactory.

E. L. Anderson.—Method 1 preferable. Method 2—unsatisfactory, except as approximate check on purity of residue.

A. W. Hanson.—No difficulty with methods. It is possible to extract with either from sodium hydroxide or ammoniacal solutions. When pyramidon hydrochloride is heated at 110°C., considerable loss occurs, and owing to variations in temperature in different parts of the oven it may not be possible to dry pyramidon hydrochloride in electric ovens unless the samples are placed together on the same shelf. Pyramidon (M-P 108) placed on the lower shelf melted but it did not melt on the upper shelf. The results submitted were dried in the water oven at 100°C.

W. F. Reindollar.—Slight tendency for dried residues to increase in weight while weighing—especially in Method 2. Suggest Method 2 be changed to use only extracted residue on account of possible impurities. Method 3 gives best results.

CONCLUSIONS.

The results of various collaborators show that both the extraction methods are entirely satisfactory, and that pyramidon may readily be extracted from either a dilute alkali or an ammoniacal solution.

Method 2 received severe criticism in most cases, and results indicate that they are justified. The irregularities appear to be due chiefly to difficulty in drying or complete removal of free hydrochloric acid. The method, however, is of some value as a check on extracted residues.

Owing to a slight tendency for dried residues to increase in weight, especially in the case of pyramidon hydrochloride, the residues should be weighed as quickly as possible. The hydrochloride will become moist on standing a short time.

RECOMMENDATION¹.

It is recommended—owing to the fact that pyramidon is readily extracted from either a dilute sodium hydroxide or an ammoniacal solution—that Methods 1 and 3 be adopted as official, or that the method be so rewritten as to make the use of either sodium hydroxide or ammonia optional.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 73.

REPORT ON MICROCHEMICAL METHODS FOR ALKALOIDS.

By C. K. GLYCART (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

In accordance with the recommendation that microchemical methods for alkaloids be further studied the work was continued.

Last year five of the more important alkaloids were studied. Control specimens and samples of unknown tablets containing salts of heroine, cocaine, morphine, codeine, and strychnine were sent to the collaborators. The results of the identification of the alkaloids in the unknown tablets were correct. However, C. H. Stephenson, Food, Drug and Insecticide Administration, Washington, D. C., advised that the work be repeated by a large number of collaborators.

Since the microchemical test for heroine (diacetylmorphine) has been adopted by the association as official¹, no work on this alkaloid was performed. Two alkaloids, pilocarpine and atropine, were included in this year's study. Directions and descriptions for the tests, control specimens, and unlabeled samples of tablets were sent to the following:

E. O. Eaton, U. S. Department of Agriculture, San Francisco, Calif.

H. Wales, U. S. Department of Agriculture, Washington, D. C.

W. F. Kunke, U. S. Department of Agriculture, Chicago, Ill.

R. S. Roe, U. S. Department of Agriculture, Chicago, Ill.

F. C. Synkovich, U. S. Department of Agriculture, Chicago, Ill.

The unlabeled samples consisted of hypodermic tablets containing alkaloidal salts, as follows: No. 1—morphine; No. 2—codeine; No. 3—strychnine; No. 4—cocaine; No. 5—pilocarpine; and No. 6—atropine.

The method submitted to collaborators has been published².

The results, descriptions, and comments of the collaborators are as follows:

E. O. Eaton.—No. 1—morphine; No. 2—codeine; No. 3—strychnine; No. 4—cocaine; No. 5—pilocarpine (by comparison with a known sample); No. 6—atropine indicated (by comparison with a known sample).

The test for the first four alkaloids as found appears to be satisfactory. I do not believe the details of the crystals or of the method are sufficient to identify pilocarpine or atropine in themselves.

H. Wales.—No. 1—morphine; No. 2—codeine; No. 3—strychnine; No. 4—cocaine; No. 5—pilocarpine; and No. 6—probably atropine.

I do not consider the test for atropine with Wagner's reagent conclusive. Under the magnification used (150X) it was impossible to determine the shape of the crystals, and no characteristic groupings were noticed.

W. F. Kunke.—No. 1—morphine; No. 2—codeine; No. 3—strychnine; No. 4—cocaine; No. 5—pilocarpine; and No. 6—atropine.

It seems to me that the atropine crystals are not sufficiently characteristic for one to be able to state definitely that atropine is present in an unknown sample which

¹ *Methods of Analysis*, A. O. A. C., 1925, 398.

² *This Journal*, 1927, 10: 371; 1928, 11: 52.

Characteristic microchemical tests for alkaloids.

Atropine	<i>Wagner's reagent</i> Small dark rods and triangular plates form in great numbers, singly and in groups.	
Cocaine	<i>Platinic chloride</i> Delicate, feathery crystals later becoming heavier in structure.	
Codeine	<i>Marme's reagent</i> Silvery circular masses, crystallizing into dark rosettes of irregular outline.	<i>Wagner's reagent</i> Heavy red-brown precipitate, crystallizing very slowly in yellow blades extending in branches (never red).
Heroine	<i>Platinic chloride</i> Spherical clusters of golden yellow needles slowly form around a nucleus.	
Morphine	<i>Marme's reagent</i> Silvery gelatinous precipitate, crystallizing in dense masses of fine needles.	<i>Wagner's reagent</i> Small drop of reagent produces heavy red-brown precipitate, slowly crystallizing in shining red overlapping plates extending in branches.
Pilocarpine	<i>Platinic chloride</i> Crystals form slowly—layers of thin yellow triangular plates of delicate structure.	
Strychnine	<i>Platinic chloride</i> Crystals form immediately in clusters and singly in small wedge-shape needles, which move about the field.	<i>Marme's reagent</i> Silvery masses slowly forming rosettes.

may contain one or more alkaloids other than the six alkaloids included in the collaborative work for this year.

R. S. Roe.—No. 1—morphine; No. 2—codeine; No. 3—strychnine; No. 4—cocaine; No. 5—pilocarpine; and No. 6—atropine.

For the most part the microscopical crystals appeared much as described in the table submitted.

Atropine with *Wagner's reagent* at first produced dense, dark, small rod-like crystals, and later coagulated to some extent in groups or bushy-like masses.

Pilocarpine with *platinic chloride* formed, rather slowly, thin plates mostly grouped in layers.

Codeine with *Marme's reagent* in addition to producing dark rosettes formed many long narrow plates grouped around a common point. Some also appeared as bundles of needle-like plates.

F. C. Synkovich.—No. 1—morphine; No. 2—codeine; No. 3—strychnine; No. 4—cocaine; No. 5—pilocarpine; and No. 6—atropine.

Codeine with *Marme's reagent* formed fine branched rosettes which became impregnated with dark dots and finally formed dark irregular masses.

The other samples were easily identified with the descriptions given.

In addition to the other tests requested, I found that *pilocarpine* with *Wagner's reagent* formed fine branches made up of small circular spots.

DISCUSSION.

The alkaloids in the unlabeled samples of tablets were identified correctly by the collaborators. However, the findings with regard to atropine were inconclusive. This may be explained in part by the fact that the control specimen of atropine consisted of powdered tablets containing only 1/100 grain instead of the pure atropine salt. It was found that the crystals formed are never large and that they form in great numbers even if 1/100 grain tablets are examined.

Wales and Kunke reported that the crystals of atropine with Wagner's reagent are not sufficiently characteristic. This was also found by the associate referee to be the case on different samples of tablets.

The results obtained by the collaborators last year¹ and also this year show that the tests for the identification of morphine, codeine, cocaine, and strychnine are satisfactory.

RECOMMENDATION².

It is recommended—

(1) That the microchemical tests and descriptions for morphine, codeine, cocaine, and strychnine be adopted as tentative.

(2) That pilocarpine, atropine, and other important alkaloids be further studied with the view to including a systematic description and diagram of microchemical methods for their identification.

REPORT ON SILVER PROTEINATES.

By LLEWELYN JONES³ (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

For the work this year further study was made of the method for the determinations of alkalinity or acidity of silver proteinates, as proposed by Eaton⁴. Some work was also done on the potentiometric titration method.

Five commercial samples representing different types and brands of silver proteinates were secured for analysis, labeled respectively: (1) "Silver Proteinate", (2) "Silver Proteinate", (3) "Vitargol", (4) "Argyrol", and (5) "Collargolum".

METHOD FOR DETERMINATION OF ACIDITY OR ALKALINITY AS PROPOSED BY EATON.

Dialyze a 1 gram sample as directed in the official method⁵, including the amendment⁶, for detection and estimation of ionizable silver compounds. Titrate with 0.02 *N* hydrochloric acid or sodium hydroxide an aliquot of the clear solution representing 0.5 of a gram sample, using phenolphthalein as an indicator.

¹ *This Journal*, 1927, 10: 372.

² For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 73.

³ Presented by C. K. Glycart.

⁴ *This Journal*, 1926, 9: 313.

⁵ *Ibid.*, 55.

⁶ *Ibid.*, 1927, 10: 46.

Samples were not sent out to collaborators. However, A. G. Buell kindly agreed to collaborate. The following results were obtained:

ANALYST	SAMPLE NO. 1 "SILVER PROTEINATE" PER CENT ACIDITY AS HCl	SAMPLE NO. 2 "SILVER PROTEINATE" PER CENT ALKALINITY AS NaOH	SAMPLE NO. 3 "VITARGOL" PER CENT ALKALINITY AS NaOH	SAMPLE NO. 4 "ARGYROL" PER CENT ALKALINITY AS NaOH	SAMPLE NO. 5 "COLLAG- GOLUM" PER CENT ALKALINITY AS NaOH
L. Jones	0.90	0.72	0.22	0.18	0.03
A. G. Buell	0.79	0.19	0.14	0.03

DISCUSSION.

Three types of silver proteinate are represented in the tabulation. Samples Nos. 1 and 2 are of the strong type, Nos. 3 and 4 of the mild type, and No. 5 of the collargol type. It is of interest to note that sample No. 1 gave an acid reaction, while sample No. 2, of the same type, gave an alkaline reaction; both samples gave positive test for ionized silver. Samples Nos. 3, 4, and 5 gave an alkaline reaction and a negative test for ionized silver.

The associate referee is of the opinion that the method proposed by Eaton gives satisfactory results and that the work done thus far is sufficient to show its value. In the dialysis method special precaution should be observed in the choice of parchment paper.

POTENTIOMETRIC TITRATION METHOD.

It was thought that the alkalinity or acidity of silver proteinates could be studied advantageously by means of potentiometric titration. For this purpose a Leeds and Northrup potentiometer was employed, the quinhydrone electrode being used against a calomel half cell containing saturated potassium chloride solution as a reference. A 0.25 gram sample was dissolved in 25 cc. of recently boiled and cooled distilled water, and the pH value was determined after the addition of each 0.2 cc. of 0.02 *N* hydrochloric acid or sodium hydroxide. In order to make a comparison with the titration method, the associate referee used 25 cc. of the dialyzed solution and determined the pH value as above.

The results obtained by this method were unsatisfactory, and for that reason they are not included in this report. Since the curves obtained by plotting the pH values against the quantity of solution used showed no definite break, it was practically impossible to determine the neutral point.

RECOMMENDATION¹.

It is recommended that the dialysis and titration method for the determination of alkalinity or acidity be adopted as a tentative method.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 74.

H. Wales: I should like to say something in regard to the potentiometric titration mentioned. Most of the silver can be determined electrolytically by the addition of a halogen. The potentials reported by Jones are due primarily to the concentration of silver ion. The addition of hydrochloric acid changes the amount of silver ion in the solution and that is what was being measured—not the pH of the solution. There was also probably some diffusion of potassium chloride from the calomel electrode to complicate matters.

I should also like to take this opportunity to mention some work which C. M. Brewer of the Insecticide Testing Laboratory and I have recently carried out in connection with other A. O. A. C. methods for silver proteins. Our problem was to determine the changes that take place when solutions of these preparations are stored. It was necessary to find suitable methods of analysis. The dialysis method proved to be unsatisfactory for other than freshly prepared solutions on account of the large amount of colored material which passed through the parchment paper. The yeast method, which was adopted as tentative last year, was rejected for reasons which will be published shortly (see p. 396). An article by Taylor¹ criticises this method rather severely. By making comparative tests of the action of a number of silver preparations against yeast and against bacteria he found that there was no relationship. One of his preparations had no inhibitory action against yeast, and yet it was most effective against bacteria.

We get the best results with the electrical titration of these compounds with potassium iodide. The changes during storage, as indicated by germicidal action, are more nearly represented by the results of potentiometric titration, although we have found that the magnitude of the titratable silver is in no sense a measure of the strength of the preparation.

A. E. Paul: I read that article of Taylor's, and it seemed to me that he showed a different effect depending on the bacteria used. That is, it would be necessary then to make a comparison for each different type of bacteria involved.

H. Wales: It is very difficult, apparently, to get consistent results. I have noticed from articles in the literature that different observers obtained fairly consistent results with gonococcus, but with *Staph. aureus* and *B. typhosus* there is no agreement.

C. K. Glycart: What is your conclusion? Is the potentiometric method the most accurate?

H. Wales: The theory of the potentiometric method is that when a silver compound is titrated with an iodide, silver iodide is formed, and all the silver which is more highly ionized than silver iodide is titrated. In a freshly prepared solution of the protargol type it is possible to

¹ *J. Am. Pharm. Assoc.*, 1927, 16: 820.

titrate nearly all the silver, but if a solution is 4 or 5 months old, the titration decreases to 60–70 per cent. If solutions of the argyrol type are used, however, only a small percentage of the silver is titrated, and the age of the solution has little effect.

There is no precipitation of silver iodide during the titration. If a little sulfuric acid is added, a precipitate is formed during titration and a clear solution is obtained. This precipitate is not silver iodide. We have reached the conclusion in our work that we are dealing with some unknown property of the silver proteins in all these methods.

C. K. Glycart: Do you think that the U. S. P. X method is reliable for these compounds?

H. Wales: It probably is, but it is too cumbersome. There has just been published a report on a number of tests to distinguish between the mild and strong silver proteinates. I don't remember which test was finally adopted, but all were much more rapid and efficient than the yeast test. You will find the report published in the last number of the Proceedings of the American Drug Manufacturers' Association.

REPORT ON TERPIN HYDRATE.

By C. W. HARRISON¹ (U. S. Food, Drug and Insecticide Administration, Minneapolis, Minn.), *Associate Referee.*

This year a collaborative study was made of the method published by A. G. Murray², for the determination of terpin hydrate in elixir of terpin hydrate.

An elixir was prepared according to the procedure outlined in the National Formulary³, 17.5 grams of terpin hydrate per liter being used. After considerable preliminary work, in which different modifications of the methods of extracting and washing were used, a procedure that followed very closely Murray's original method was selected. The details of the method were sent to collaborators with samples of the elixir.

The method is as follows:

METHOD FOR DETERMINATION OF TERPIN HYDRATE IN ELIXIR OF TERPIN HYDRATE.

REAGENTS.

Salt solution.—Dissolve 20 grams of common salt in water and make to 100 cc., or dilute 3 volumes of saturated salt solution with 1 volume of water.

Alcohol-chloroform.—Chloroform containing 5–7 per cent by volume of alcohol.

DETERMINATION.

Measure 10 cc. of elixir of terpin hydrate into a separatory funnel. Dilute with 25 cc. of salt solution. (The quantity of terpin hydrate to be weighed should be approxi-

¹ Presented by A. G. Murray.

² *This Journal*, 1927, 10: 257.

³ National Formulary, 5th ed., 1926, p. 48.

mately 0.2 gram and the dilution with salt solution should be such as to reduce the alcoholic content to about 10-15 per cent by volume before extracting with alcohol-chloroform.)

Extract successively with six 15 cc. portions of alcohol-chloroform, separating the chloroform layer carefully each time so that none of the watery layer will be carried through with the chloroform.

Collect all the chloroform fractions and wash twice with salt solution, using 15 cc. for the first washing and 5 cc. for the second. Wash each salt washing with a 5 cc. portion of alcohol-chloroform, adding this portion to the original chloroform extracts.

Filter the combined chloroform extracts containing the terpin hydrate in solution through a plug of cotton into a small, low-form, tared beaker (100 cc. size), being sure that the separation from salt wash water is perfect. Evaporate off the chloroform at room temperature with the aid of an air blast. Use no heat in the evaporation. Wipe off the beaker when the chloroform is entirely gone, allow to stand in the balance for 10 minutes, and weigh.

Do not dry in a desiccator because terpin hydrate loses water under these conditions. Report in terms of grams per 100 cc.

The results obtained are given in the following table:

COLLABORATOR	GRAMS PER 100 CC.	
T. F. Pappe Baltimore, Md.	1.776	} 1.784
	1.794	
	1.782	
H. H. Mattern Baltimore, Md.	1.785	} 1.788
	1.791	
C. W. Harrison	1.775	} 1.777
	1.770	
	1.752	
	1.812	

It is shown in the tabulation that when the method is applied to this preparation of terpin hydrate, the results, on the average, are about 2 per cent too high. The method is open to the serious criticism that it is a determination of chloroform extract and that it would not be applicable to determinations of terpin hydrate in mixtures containing other chloroform-soluble extractives which might be removed by alcohol-chloroform under these conditions and not removed by the salt water washing or spontaneous evaporation. Aside from these limitations, the method has considerable value and is worthy of retention and further study with a view to extending its usefulness to other preparations of terpin hydrate.

RECOMMENDATIONS.¹

It is recommended—

(1) That the Murray method as modified by the associate referee be studied further, as well as any other method which may be available for the determination of terpin hydrate.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 74.

(2) That the liquid extractor described by Palkin and Watkins be tried as a means of removing terpin hydrate from its solution and that a variety of solvents be tested in connection with this apparatus.

No report on santonin was given by the associate referee.

REPORT ON ETHER.

By G. C. SPENCER (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The quantitative estimation of ether in admixture with alcohol and water has been attempted more or less successfully by a number of chemists, most of whom have based their procedures on the quantitative oxidation of the ether by strongly acidulated potassium dichromate under stipulated conditions.

The procedure outlined by Somogyi¹ was given considerable study during the past year. It directs the introduction of the ether and alcohol vapors contained in a known weight of sample into a stream of dried air with which the vapors are bubbled successively through a sulfuric acid solution (1-3) and through a mixture of concentrated sulfuric acid and normal potassium dichromate solution in equal parts by volume. The sulfuric acid in the first tube retains the alcohol and permits the ether vapors to pass through into the second tube where the ether is oxidized. By causing the alcoholic solution in tube I to react with an excess of standard potassium dichromate solution and titrating this excess, the quantity of alcohol is readily estimated. Likewise, the excess of potassium dichromate remaining in tube II can be titrated, and the weight of ether represented by the difference is easily calculated.

Repeated trials have demonstrated that the method outlined by Somogyi is not dependable for such mixtures as may be obtained by distillation from medicinal preparations, although it may give satisfactory results for known weights of anhydrous alcohol and ether.

After considerable experimentation the original Somogyi method was modified to read as follows:

REAGENTS.

Sulfuric acid solution.—Mix 1 volume of concentrated sulfuric acid with 3 volumes of water.

Concentrated sulfuric acid.

Normal potassium dichromate.

0.1 N potassium dichromate.

0.2 N ferrous ammonium sulfate.

Diphenylamine indicator.

¹ *Z. angew. Chem.*, 1926, 39: 280.

PROCEDURE.

Set up the apparatus described by Somogyi (loc. cit.) with the following modifications:

Provide both tubes, I and II, with jacket tubing by means of which No. I may be kept at the temperature of 50°C. and No. II at the temperature of ice water. Add 150 cc. of sulfuric acid solution to tube I. Mix 50 cc. of normal potassium dichromate with an equal volume of concentrated sulfuric acid and after cooling transfer the mixture to tube No. II.

Weigh 0.3–0.5 gram of the distillate to be examined in a small glass-stoppered capsule. Introduce the capsule into flask *a*, close the flask, and start the current of air through the train. Warm flask *a* by immersing it in hot water until the glass stopper of the capsule is expelled. Continue the aspiration of air through the apparatus until all the liquid under examination has been carried into the absorption tubes. Two hours has been found sufficient to complete the reactions under the conditions stated above.

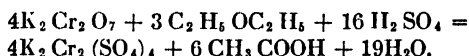
(The contents of tube I may be used for the estimation of alcohol, but no report on this phase of the work will be made at this time.)

Dilute the contents of tube II and make up to a volume of 250 cc. Use a 10 cc. aliquot for titration, as follows:

Dilute the aliquot to a volume of 100 cc. and add 10 cc. of 0.2 *N* ferrous ammonium sulfate. Titrate the excess of ferrous iron with 0.1 *N* potassium dichromate solution by the method of Knop¹, using diphenylamine as indicator.

Calculate the weight of ether oxidized from the quantity of potassium dichromate decomposed. One cubic centimeter of normal potassium dichromate corresponds to 0.0009256 gram of ether.

The chemical reaction involved is stated as follows:



The results obtained by this method are given in the following tables:

Ether alone.

(1 cc. 0.1 *N* $\text{K}_2\text{Cr}_2\text{O}_7$ = 0.0009256 gram of ether.)

ETHER	0.1 <i>N</i> $\text{K}_2\text{Cr}_2\text{O}_7$ CONSUMED	FOUND	RECOVERED
<i>gram</i>	<i>cc.</i>	<i>gram</i>	<i>per cent</i>
0.3066	327.6	0.3060	99.8
0.2175	233.6	0.2162	99.4
0.3665	397.5	0.3679	100.4
0.3210	352.2	0.3260	101.5
0.2731	293.7	0.2718	99.5
0.2576	278.3	0.2557	99.2
0.2496	269.7	0.2492	99.8

Ether-alcohol-water mixture.

SAMPLE	ALCOHOL ADDED	WATER ADDED	ETHER ADDED	0.1 <i>N</i> $\text{K}_2\text{Cr}_2\text{O}_7$ CONSUMED	ETHER RECOVERED
<i>gram</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>cc.</i>	<i>per cent</i>
0.4275	42.5	17.35	40.15	189.2	40.96
0.5000	42.3	25.71	31.97	168.2	31.14
0.5893	37.24	27.59	25.17	226.0	35.5

¹ *J. Am. Chem. Soc.*, 1924, 46: 263.

The associate referee wishes to acknowledge his indebtedness to H. M. Joslin for valuable assistance and for suggestions for improving the Somogyi method.

RECOMMENDATIONS¹.

It is recommended that work on the Somogyi method for ether be continued throughout the coming year with special reference to medicinal preparations and that if possible the method be referred to collaborators.

REPORT ON BIOASSAY OF DRUGS.

By J. C. MUNCH (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

CAT-EYE METHOD.

The bioassay method for the quantitative determination of mydriatics reported last year² and adopted as a tentative method was used during the past year. Collaborators report that the method proved satisfactory. For simplification of technic, it was deemed advisable to continue the use of the ordinary 1 cc. Mohr pipets, which are readily available, rather than to devise a special pipet or syringe for these assays.

This method was studied extensively to determine its suitability for the assay of myotics. G. S. Gittinger's report gives the concentrations statistically found to be the average minimum effective concentrations. Cats were handled under the same conditions for assaying myotics as for assaying mydriatics. The effect came on in about the same time.

The method has been published³.

REPORTS AND COMMENTS OF COLLABORATORS.

G. S. Gittinger, Pharmacology Laboratory, F. D. I. Administration.—Further experience with this method tends to confirm the thresholds found in Munch's previous work. The method proves valuable in the assay of mixtures such as morphine-atropine, as well as in the study of the conversion of hyoscyamine to atropine. It is also of value in the study of unknown mixtures.

This method has been used for the quantitative determination of various myotics. For such work the technic employed is essentially that given in the tentative method. No special comments are needed for the preparation of animals or of solutions. Cats are placed one foot from a 100-watt electric lamp, and the maximum contractibility of their pupils is determined just before the application of myotic solutions. At various intervals (usually 1–2 hours), the cats are placed in the same position, and differences in the pupillary diameters are as readily discernible following myotics as following mydriatics.

Unless the concentrations employed were greatly in excess of the minimum effective concentrations, the pupils returned to normal within 24 hours following the use of

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 74.

² *This Journal*, 1927, 10: 383.

³ *Ibid.*, 1928, 11: 53.

either mydriatics or myotics. No clear-cut evidence of consensual mydriasis or myosis was obtained during these experiments. Some experiments suggested the possibility that the application of a myotic in doses materially in excess of the minimum effective concentration might produce some degree of consensual mydriasis. With the very large doses of duboisine there was some evidence of consensual myosis. However, this was not followed in detail.

Ninety different products were tested for their effect upon the pupil of the cat's eye. As a preliminary or qualitative test, approximately 1-2 mg. of the crystalline material or 1 drop of a liquid was dropped directly upon the cornea. The lids were gently tracted and retracted until the material was completely dissolved. Care was taken to prevent drainage of this solution from the cornea into the inner canthus. Examinations were made after 1 or 2 hours, and occasionally after longer periods of time. In case either mydriasis or myosis was noted, solutions of known concentrations were tried, and the minimum effective concentration was determined in accordance with directions outlined in the tentative method. The minimum effective concentration of atropine was 12 mg. per liter. One drop of this concentration contained 0.0006 mg. Accordingly, it may be seen that the application of 1-2 mg. of these drugs in this qualitative test gave a total quantity of drug 2000-4000 times as much as the minimum effective concentration of atropine. It was believed that unless this quantity showed some evidence of physiological activity quantitative methods of assay were not indicated.

L. W. Rowe, Parke-Davis Company.—Further experiments have been conducted in our laboratories on the cat-eye method of testing mydriatics to determine the thresholds of four cats for scopolamine and for atropine. The thresholds found are as follows:

ALKALOID	CAT NO. 1	CAT NO. 2	CAT NO. 3	CAT NO. 4
Scopolamine	Below 0.5	0.4	0.8	Between 0.4 and 0.8
Atropine	8	10	Below 12	Below 15

These results are in terms of milligrams of alkaloid per liter of solution.

Cat No. 1 was very susceptible to both alkaloids; cats 3 and 4 seemed to be more susceptible to atropine than to scopolamine. Two experiments were conducted on mixtures of scopolamine and atropine. Cat No. 3—0.25 mg. per liter of scopolamine and 0.055 mg. per liter of atropine—pupil only dilated about $1\frac{1}{2}$ times normal in 1 hour and was normal in 5 hours. Cat No. 4—0.40 mg. per liter of scopolamine and 0.1 mg. per liter of atropine—gave a slight effect in $1\frac{1}{2}$ hours and was still slightly effective after 5 hours. These results were so erratic, and cat No. 3, which was thought from other data to have a higher threshold, reacted appreciably to a dose so far below the threshold dose that it did not seem advisable to test other combinations.

One solution of scopolamine was made up as an unknown and submitted to an assistant for test. He reported that 1 : 25 solution had a slight effect on cat No. 1, and that a 1 : 50 solution had no effect on cat No. 2. A 1 : 25 solution seemed to be the threshold test and this corresponded to 0.4 mg. of scopolamine per liter, which is the threshold reported. However, cat No. 1 is the most susceptible, so a lower threshold dose should have been found on this cat.

Summarizing very briefly, we believe (1) every cat must be thoroughly tested to determine its individual threshold; (2) that mixtures of mydriatics cannot be so easily tested and calculated as was to be expected.

Carl Nielsen, Abbott Laboratories.—In the early part of 1927 various discussions and experiments concerning a method to measure and record the size of pupil dilation took place among Nielsen, Spruth, and Cannon. After several devices had been tried it was concluded that comparison of the treated eye with the untreated one at the point of maximal pupil contraction produced under constant conditions was the most satis-

factory method for determining a minimum perceptible mydriasis. The human eye under these conditions can easily distinguish a difference of 0.5 mm. or less in the size of the cat's pupil.

The constant condition used for observation was brought about by placing the cat in a light-proof box 27" x 19" x 15". One 27" x 19" side was open. Over this open side a 2-yard focusing cloth was draped. Inside of the box a 100-watt bulb mounted in a desk lamp was placed.

When the box was used the operator placed the cat in the box so that its eyes were directly in front of the lamp and twelve inches from it. The focusing cloth was draped over the operator's head and shoulders and thus all light was excluded except that which shone directly into the cat's eyes from the 100-watt lamp. In all other respects the Department of Agriculture technic was followed exactly.

In February and March of 1927, Cannon found that 0.05 cc. of a solution containing 0.4 mg. of hyoscine per liter, as contained in our H. M. C. No. 1 tablets x-3, produced a perceptible dilatation, while 0.05 cc. of a solution containing 0.35 mg. per liter did not; 0.05 cc. of a solution of hyoscine hydrobromide such that 1 liter contained 0.4 mg. produced a perceptible mydriasis, while a similar solution containing 0.3 mg. per liter also produced a perceptible mydriasis. The Department of Agriculture found the minimum dose to be 0.05 cc. of a solution containing 0.4 mg. per liter.

In August, 1927, I found the atropine content of a routine morphine and atropine compound x-2 sample to be correct according to the formula accepting the marginal dose of atropine as in March, 1926.

In all this work the same cat was used. At least a 48-hour rest was given between tests.

The pressure of more important problems prevented further work on this one.

H. R. Walkins, F. D. I. Administration.—The use of the cat-eye method upon approximately 100 solutions obtained during the course of my experiments upon the stability and character of various alkaloids extracted from hyoscyamine by a mechanical extractor, have proved that this method is of great value for the assay of these mixtures. Experiments were also conducted with dried lettuce treated by the mechanical extractor. The dried leaves were found to contain approximately 0.03 per cent of alkaloids. Tests upon the cat eye, however, show that they were physiologically inert.

The following results were obtained by Gittinger:

Cat-eye results.

Mydriatics: Minimum effect concentration: mg./liter. All results were calculated in terms of the alkaloids.

Acetanilid, acetphenetidum, acetylsalicylic acid, aconitine, allonal, alypine, amytal, anesthesine, antipyrin, apomorphine HCl, and aposthesine—no effect. Arecoline HBr¹—10,000 and atropine sulfate—12.

Barbital, bebeerine, berberine, brucine sulfate, butesin, and butyn—no effect.

Caffeine, cephaeline HCl, chloretone, chloral hydrate, cincophen, cinchonidine sulfate, cinchonine sulfate, cinchotoxine HI, colchicine, cotarine HCl, and curare—no effect. Cocaine HCl—60.

Dionine HCl, digitalis (German), digitonin, and digitoxin—no effect. Daturine—12 and duboisine—1.6.

Elaterin, emetine, epinephrin 1 : 1000, eucatropine, eucupin, ergotoxine phosphate, and eumydrine—meth. atrop. nitrate—no effect. Ephedrine sulfate—2000, pseudoephedrine sulfate—4000, and euthalmin HCl—2500.

Formalin (1 : 100)—no effect.

¹ Myotic.

Gelsemine resin and gelseminine HCl—no effect.

Heroin, holocaine, and hydrastine—no effect. Homatropine HBr—10, hyoscine—0.4, and hyoscyamine—4.0.

Ipral—no effect.

Lettuce (F. E.) and luminal—no effect.

Methyl atropine bromide—400.

Narceine HCl, narcotine HCl, nicotine ZnCl₂, novocaine, and numoquin—no effect.

Papaverine, piperine, and psicaine—no effect. Physostigmine¹—10 and pilocarpine¹—25000.

Quercitrin, quinidine sulfate, quinotoxine HI, and quinine sulfate—no effect.

Salicaine, santonine, saponin, sapotoxine, and solanin—no effect. Scopolamine (see hyoscine).

Sparteine sirup, stovaine, strychnine sulfate, and sulfon-methane (sulfonal)—no effect.

Theobromine, theophylline-sodium, tropacaine, and tutocaine—no effect.

Urethane—no effect.

Veratrine—no effect.

Yohimbine HCl—no effect.

RECOMMENDATIONS².

It is recommended—

(1) That the cat-eye method adopted in 1927 as a tentative method for the assay of mydriatics be also adopted as a tentative method for the assay of myotics.

(2) That the cat-eye method be adopted as an official method of assay.

COLLABORATIVE ASSAY OF TINCTURES OF DIGITALIS.

By the U. S. P. X—1-Hour Frog Method.

No large-scale cooperative assays to determine, specifically, the accuracy of the U. S. P. X 1-hour frog method for the assay of tincture of digitalis have come to the attention of the associate referee, although some collaborative determinations were conducted by the American Drug Manufacturers Association in 1922 and 1923. Twenty-eight collaborators, representing practically all the drug manufacturers and the consulting bioassayists of the United States, agreed to participate in an A. O. A. C. cooperative study of three tinctures of digitalis.

PREPARATION OF SAMPLES.

Twenty-five pounds of bioassayed digitalis leaves were obtained from a prominent American importer for use in this study. Analysis by J. F. Clevenger of the Pharmacognosy Unit of Drug Control showed that the material contained 10 per cent of total ash and 0.1 per cent of acid-insoluble ash. The number of brown and dried leaves was well within the pharmacopeial limit, and the sample was passed as being in accordance with U. S. P. requirements. A physiological assay showed that this material was 120 per cent of the U. S. P. requirements for physiological activity. A portion of this shipment was ground, and a tincture was prepared by

¹ Myotic.

² For report of Subcommittee B and action of the association, see *This Journal*, 1928, 11: 53.

following in detail the method outlined in U. S. P. X. The volume of percolate was such that 10 cc. of finished percolate represented 1 gram of crude digitalis leaves. This tincture was physiologically assayed and found to be 120 per cent of U. S. P. requirements. This sample was marked Tincture B. By the addition of 71 per cent alcohol (the concentration specified in U. S. P. X for the preparation of tincture of digitalis) one portion of Tincture B was diluted to 100 per cent U. S. P. physiological potency and labeled Tincture C. Another portion was diluted to 80 per cent U. S. P. physiological activity and labeled Tincture A. The pH of these tinctures was determined by B. A. Linden of the Microbiological Unit of Food Control Laboratory by means of the quinhydrone electrode and found to be—

Tincture A—5.01.

Tincture B—4.996.

Tincture C—4.996.

U. S. P. X standard ouabain (150 mg.) was dissolved in 150 cc. of 70 per cent ethyl alcohol, and 5 cc. portions were sealed in Pyrex ampoules.

A set of samples consisting of 100 cc. of each tincture and of 5 cc. of ouabain solution were sent to each of the 28 collaborators on June 3, 1927. Arrangements were made whereby each collaborator would conduct assays of these products during the period between June 15 and June 30, 1927. This period was specified in order to avoid variations due to different rates of deterioration and to differences in susceptibility of frogs at various seasons of the year. However, for one reason or another, some collaborators were unable to make these assays during the indicated period.

RESULTS.

Results obtained by 21 collaborators are given in Table 1. The comments and criticisms submitted by various participants indicate the general trend of opinion as to the accuracy of the 1-hour frog method for the assay of digitalis. The results have been considered under two general headings: (1) The absolute values obtained; (2) the relative values obtained. Under the heading, "Observed Values M. S. D.," the experimental values reported by each collaborator are given. The figures in parentheses represent the number of frogs used for each assay.

It may be noted that the value for ouabain ranges from 0.4 to 0.75 mg. per kilo. However, the results obtained by each collaborator in his own laboratory are consistent, irrespective of the value found. This tends to prove that the frog and not the ouabain is the physiological variable concerned.

The three columns under the general heading "Corrected Values, as Percentage, U. S. P. X Tincture Digitalis" give the results obtained by each collaborator when calculated as percentages of the physiological

potency for U. S. P. X tincture of digitalis. The standard frogs have a minimum systolic dose of 0.5 mg. per kilo, and these figures have been obtained by calculating the preparations of the different tinctures in terms of a value of this magnitude for the frogs. It may be noted that there is a fairly large discrepancy in the results obtained by different collaborators. These discrepancies are much larger when the collaborators failed to follow the U. S. P. X procedure carefully, but some of them are due to the fact that the assays were conducted by relatively unskilled workers.

In order to determine the absolute value of a tincture of digitalis, it seems evident that the method of assay outlined in U. S. P. X *must* be followed carefully and that the procedure be conducted by an analyst with a reasonable degree of experience in the use of this method.

In order to obtain information upon the relative values of Tinctures A, B, and C, Table 2 was prepared. Knowing that these tinctures of digitalis were so prepared that Tincture A was 80 per cent, Tincture B—120 per cent, and Tincture C—100 per cent of the U. S. P. X physiological standard for tincture of digitalis, the associate referee determined deviations of "observed" values from the "true" values. It is noted that in the hands of analysts who are following U. S. P. X procedure with reasonable care, these deviations range from 0 to 20 per cent. In the hands of analysts who are not sufficiently experienced or who are not following the U. S. P. method carefully, much greater deviations are noted. The average deviation of all values is 12.6 per cent, and the probable error 10.6 per cent. The mean value found, 7.5 per cent below the true mean, shows that the general tendency was toward too low results. These results when considered statistically show that bioassayists following the U. S. P. X 1-hour frog method with a reasonable degree of care may be expected to obtain results within plus or minus 10 per cent of "true" values, at least half of the time. Consideration of the results obtained by 10 analysts most thoroughly acquainted with and regularly using the U. S. P. X method gave a probable error of 5 per cent. Results by nine analysts who were less familiar with the method showed a probable error of 8.5 per cent. Inadequately trained workers will have less chance of obtaining concordant results, but with care they should check within plus or minus 10 per cent.

No consistent increase in accuracy was noted with an increase in the number of frogs used. Some workers who used less than 50 frogs obtained results which were in remarkable agreement with the "true" values, whereas other workers who used five times as many frogs obtained much greater deviations. This indicates again that adequate training in the use of the 1-hour frog method is a necessary prerequisite to the obtaining of satisfactory assay results. However, it is advisable to use at

TABLE
Collaborative results of assay

ANALYST NUMBER	DATE OF EXPERIMENTS, 1927	SOURCE OF FROGS	WEIGHT OF FROGS	TEMPERATURE	NUMBER FROGS USED	APPROXIMATE CONCENTRATION ALCOHOL TINCTURE DIGITALIS INJECTED	DEVIATIONS FROM U. S. P. X SPECIFICATIONS
1*			gram	°C.		per cent	
2	6/21-28	Indiana	10-28	15	81	20	Many frogs under U. S. P. weight.
3	6/30	Michigan	6.5-15	20 ^p	57	24-35	All frogs under weight. Analyst not accustomed to 1-hour frog method.
4*	6/23-24	Illinois	14-29.5	15-17	32	35	Results obtained at irregular intervals greater than 1 hour.
5	6/30	Illinois	14.5-30	20	52	24	
6	6/14-21	Illinois	8-23	20	124	35 and less	Majority of frogs under weight.
7	6/30	New York	14.5-26.5	18	32	24	Only 1 frog under weight.
8	6/21-29	Province of Quebec	20-40	20	126	20-28	Only a few frogs over weight.
9	6/21-7/7	Indiana	13-32	20-25	131	20-42	Only a few frogs under weight.
10	6/28	Illinois	9-17	20	51	20	Stored 23°-28°C. Injected through leg. All frogs under weight.
11	6/20-27	Illinois Vermont	19-29 16-20	20	45 56	35	Stored 22°-23°C.
12	6/21	Illinois	18-34	23	162	42	Temperature 23°C.
13	6/7-30	Vermont	10-31	17-21	179	24	Ouabain value not determined. Most frogs under weight.
14	7/18	Illinois	12-31	20	41	20	
15	7/19	Pennsylvania	15-43	20	46	35	
16	6/15-8/1	Illinois	19-33	20	426	20	Operator somewhat inexperienced.
17	6/15	Wisconsin and Illinois	18-36	20	267	20	
18	6/18/27	Illinois	18-32	20	210	20	Determined dose causing SS in $\frac{1}{3}$ frogs injected.
19	August		25-36	^p	17	^p	Ouabain value not determined.
20	June and August	Illinois	18-31	20	60	28-45	
21	6/15	Indiana and Wisconsin	12-37	20	252	24-35	

* Collaborator did not follow U. S. P. X 1-hour frog method.

1.

of tinctures of digitalis.

OBSERVED VALUES M. S. D.				CORRECTED VALUES, PERCENTAGE, U. S. P. X TINCTURE DIGITALIS		
Ouabain cc./kg	Tincture A cc./kg	Tincture B cc./kg	Tincture C cc./kg	Tincture A	Tincture B	Tincture C
(28) 0.65	(23) 8.7	(14) 6.0	(16) 7.1	90	130	110
(15) 0.6	(12) 12.0	(15) 9.0	(15) 10.0	60	80	72
(8) 0.61	(8) 7.5	(8) 16.0	(8) 13.0	98	46	56
(6) 0.5	(19) 5.5	(14) 5.0	(13) 5.2	109	120	115
(18) 0.7-0.75	(49) 8.4	(39) 5.6	(18) 6.5	100	150	130
(7) 0.49	(10) 6.5	(7) 5.0	(8) 5.5	90	120	105
(27) 0.47	(43) 8.7	(29) 6.4	(27) 7.3	65	88	77
(29) 0.5-0.55	(36) 9-9.5	(30) 6-6.5	(36) 9.5-10	65	100	80
(12) 0.7	(15) 11.0	(12) 7.0	(12) 8.5	76	120	100
(11) 0.45	(11) 8.5	(10) 5.5	(13) 6.5	64	98	84
(11) 0.4	(16) 7.5	(14) 5.0	(15) 6.0	64	97	80
(38) 0.84	(45) 13.2	(41) 9.5	(38) 12.0	76+	106+	84
—	(69) 7.0+	(56) 6.5	(54) 6.5	—	—	—
(9) 0.4	(11) 7.0	(9) 5.0	(12) 6.0	68	96	80
(10) 0.55	(12) 10.0	(10) 6.5	(14) 9.0	66	102	73
(134) 0.35-0.55	(136) 5-8	(84) 5.0	(72) 5.0	83	97	84
(32) 0.5	(62) 5.5	(66) 5.0	(107) 10.0	109	120	60
(62) 0.6-0.63	(45) 10-11	(58) 6-6.5	(45) 8-8.5	70	118	90
—	(5) —	(6) —	(6) —	70	130	115
(15) 0.55-0.6	(15) 10	(15) 9	(15) 12	72	80	60
(58) 0.6	(64) 8.5	(65) 6.0	(65) 7.5	81	115	92

least 25 frogs for the assay of each preparation, and as many more to determine the ouabain value.

No consistent difference was noted in the reaction of frogs obtained from different parts of the country. Some workers used frogs which were smaller than the 20–30 gram weight range prescribed in U. S. P. X. The results obtained in many instances were remarkably close to the “true” results. It would accordingly appear that smaller sized frogs might be used for this assay. These problems are being studied in this laboratory.

Some workers obtained very discordant results, but in observing the reports submitted it is noted that the samples injected contained much more than the 20 per cent of alcohol specified in U. S. P. X. In some instances as much as 45 per cent alcohol was present in the solutions injected. Accordingly, it appears that attention should be directed to the alcohol content of the solution actually injected into the frog.

TABLE 2.

Relative accuracy of collaborative analyses of tinctures of digitalis by U. S. P. X 1-hour frog method.

DEVIATION FROM TRUE VALUE					COMMENTS
Analyst No.	Tincture A	Tincture B	Tincture C	Average Deviation	
EXPERIENCED ANALYSTS.					
2	10	10	10	10	J. C. M. interpretation of data submitted.
5	29	0	15	15	
6	10	5	0	5	
7	10	0	5	5	
10	4	0	0	1	
12	4	14	16	11	
17	29	0	40	4	
18	10	2	10	7	
19	10	10	15	5	
21	1	5	8	4	
INEXPERIENCED ANALYSTS.					
3	20	40	28	30	Prefers another method.
6	20	30	30	27	Not in U. S. Little experience.
8	15	32	23	23	
9	15	20	20	18	
11	16	22	16		Prefers another method. New apparatus.
11	16	23	20	19	
14	12	24	20	19	
15	14	18	27	20	
16	3	23	16	2	
20	8	40	40	29	

Each collaborator was requested to follow the U. S. P. X 1-hour frog method in utmost detail, and also to report the results obtained by his regular method. Only one collaborator reported upon the assay of ouabain and of the three tinctures by a method other than the 1-hour frog method, so no conclusions as to the relative accuracy of the different methods are warranted. This problem is also being studied in this laboratory.

CONCLUSIONS.

When the U. S. P. X 1-hour frog method is followed in detail by a careful and experienced analyst, the probable error of results obtained is plus or minus 10 per cent.

COMMENTS OF COLLABORATORS.

Frederick C. Atkinson, 213 E. South St., Indianapolis.*—Absorption was extremely poor in the case of many tests on the tinctures. In fact, it was not possible for us to get good absorptions until we procured the third lot of frogs and these were given no food for 7–10 days before the tests. As to whether the matter of food had any effect on absorption we cannot say. This last lot of frogs was kept in practically winter storage for 2–3 weeks. They were free from disease. The question arises as to whether the tinctures contained some substance which retarded absorption but which later deteriorated in its effect. Ouabain solution was properly absorbed.

D. C. Beach, Norwich Pharmacal Co., Norwich.*—It is our practice to inject a pair of frogs at each dose. We take as the end point that dose which stops the heart of both frogs in ventricular systole. The next lower dose should leave both hearts beating at least weakly. Usually in our work as a check after a satisfactory end point has been determined three additional pairs of frogs are injected, one pair at the end point, one 10 per cent higher and one 10 per cent lower. Using all possible care, we find that a certain number of frogs, approximately 1 of 3 or 4, will not respond regularly, and the results must be discarded from the series. Interpretation of the results is of course an important factor in bioassay work. These methods do not give absolutely definite results in the same manner as chemical assay methods. No doubt there is some variation possible owing to the interpretations of individual operators. I have studied somewhat in detail the tabulation of results which you submitted and was certainly surprised at the extreme variations obtained by different workers.

W. H. Blome and H. G. Derthick, Frederick Stearns and Co., Detroit.*—In respect to our variation in temperature we have no means of maintaining a constant temperature other than with running water. The water from our city supply, while fairly constant, is bound to show some variation with weather temperature.

Mortimer Bye, Wm. S. Merrell Co., Cincinnati.*—Our operator reports that he experienced some difficulty in getting frogs of as good quality as we have been accustomed to using. I find that my analyst's greatest variation from your standard is 15 per cent and considering that this assay was made hurriedly and under pressure, I feel that the results were not so terribly bad, especially when compared with the other reports. I note that there are relatively few of the reports which are better than ours, and this record will make us that much more anxious to be perfect on the next series of tests run.

Edgar B. Carter, Swan-Myers Company, Indianapolis.—We have studied this chart from a number of different angles, but have been unable to arrive at any conclusions other than the fact that either the frogs or the operators will have to be standardized.

* Active collaborators.

Certainly Analyst 10 is a good man, and there are a few things that could be said for Analyst 2.

Guy W. Clark, *Lederle Antilozin Laboratories, Pearl River*.—A wide variation is to be expected, I suppose, in the frog assay.

E. Fullerton Cook, *Chairman, Revision Committee, U. S. P.*—Thank you for the study of the assay of tincture of digitalis. I will place it before the General Committee. Such contributions are of great value to the work of the next revision. One may, of course, argue either way from the results. After all, experience and practice is of great importance, but of course a uniform method must first be available. It seems to me that you are in a position to be a clearing house for difficulties of all manufacturers who may carry out biological standardization methods in the United States and that your reports will be of especial value in the next revision. As soon as you complete any study, I shall be glad to publish it in the U. S. P. circulars.

C. W. Edmunds, *Department of Materia Medica and Therapeutics, University of Michigan Medical School, Ann Arbor*.—I think the most evident lesson to be learned from the study is that there is some very poor work in bioassays being done over the country, and this seems to me to be a very serious matter. There are, on the other hand, some very good results in this list—for instance, assays 2, 7, 10, and 21, as well perhaps, as some others, seem to be very satisfactory.

Frankly, I do not think that anything can be told from the study as copied in this report about the accuracy of the U. S. P. method. We must have information, it seems to me, concerning the men who did the work, and you doubtless have this. I should like to know the individual assays that were carried out by men whom you would consider competent, and see how their results agreed, and that would evidently eliminate several of these reports.

There is one lesson, it seems to me, to be drawn from this study. It is evident that a number of men who are doing this work were not trained for it, and since receiving your letter I have wondered as to the possibility or practicability of furnishing instruction to these men. Do you think that if a short intensive course were to be offered in this laboratory, lasting say, two weeks, that the firms who are engaged in bioassay work, and whose men are clearly incompetent, would be willing to send their men here for such a course? If you thought it would be worth while, I believe that we could arrange for such instruction, and personally I think it would well repay the effort. Of course, this is only a tentative suggestion, and we have not thought the matter out carefully, but I believe it could be arranged. I do not think the expense to the manufacturers would be great, and I think they would be well repaid for the outlay. Of course men engaged in this work who are adequately trained and supervised would not wish to come.

Thos. S. Gilhens, *H. K. Mulford Co., Philadelphia*.*—The purpose of the standards provided by the U. S. P. is to give some assurance that products of identical label obtained from different sources shall be of approximately the same strength. The value of a test from the standpoint of inclusion in the U. S. P. is therefore determined by the question whether different persons or laboratories working with the test can obtain like results. From this point of view the results submitted in the table may be considered under three heads—

1. The possibility of assaying digitalis directly on the frog.
 2. The possibility of standardizing a given lot of tincture of digitalis against ouabain.
 3. The possibility of standardizing a given lot of tincture of digitalis against another lot of tincture of digitalis.
1. The wide variation in response of different lots of frogs is well brought out by

* Active collaborators.

the table, the observed systolic doses of the standard tincture (C) varying from 5.2 cc. per kilogram to 12 cc. per kilogram. This confirms previous reports and shows the unreliability of the unstandardized frog as an animal for quantitative digitalis assay.

2. Unfortunately, the results are very little better when each lot of frogs is "standardized" against ouabain. As far as they go, they show the extreme difficulty of assaying tincture of digitalis (and presumably other digitalis preparations) on the frog in terms of ouabain. Of the 21 analysts, 18 compared the tinctures to a standard solution of ouabain. One analyst (6) found the latter 9 times as active as the standard tincture (C), another (20) found it 20 times as active. The average proportion was 1 : 14 $\frac{1}{2}$. Only 6 of the analysts found values within 10 per cent of this figure (between 13 and 15 $\frac{1}{2}$). The average variation from average was almost 20 per cent, and no particular tendency was seen to approach the average; thus 3 of them fixed on a relation of 1 : 11 and 2 of them on 1 : 20, both figures near the extreme variation.

3. The results obtained by comparing the three tinctures among themselves were much better. When the results given in the table of "Corrected values, as percentages" are so adjusted that C is expressed as 100 per cent, then B should have a value of 120 and A of 80. Of the 18 laboratories using approximately the U. S. P. method, 7 had results for both A and B within 5 per cent of the theoretical figures and 5 others found values for both within 10 per cent. The figures for B are quite good, only two analysts deviating more than 10 per cent from the proper proportion to C, and all reporting it distinctly stronger. For no evident reason, the results for A were not so exact.

1. The extreme variation in the "Observed values" shows the unreliability of the "unstandardized" frog as an animal for quantitative digitalis assay.

2. The wide variation in the "Corrected values" indicates the unreliability of attempts to standardize one drug of the digitalis series in terms of another.

3. The relatively exact determination of the values of the three digitalis tinctures in relation to one another indicates that a satisfactory assay for digitalis on the frog might be obtained if the "standard" were also made from digitalis. These results recall those of Burn (alluded to in my letter of July 25) who found it impossible to standardize different lots of squill against ouabain (on frog and on cat) but obtained constant results on comparing them with a "standard solution" of scillaren.

4. The results obtained by those laboratories which followed the U. S. P. method closely (7, 8, 9, 14, 15, 16, 17) were no better than the results of those that used underweight frogs and stored at too high a temperature (2, 3, 6, 10, 11, 12).

*R. I. Grantham, Sharp and Dohme, Baltimore.**—In regard to the frogs, I do not consider it of prime importance that all frogs should fall within the scope of 20–30 grams. I do think that they should be of uniform weight, as near as it is possible to obtain them. Since the assay of tincture of digitalis by the 1-hour frog method is in reality a comparison of the activity of the tincture with that of ouabain, it seems to me that the condition of the frogs is the most important factor to be observed. I do not think that frogs which are not in a normal state of health should be used.

The amount of alcohol present in tincture of digitalis may or may not be disregarded in so far as the toxicity or physiological action of the alcohol is concerned, and it is quite logical to assume that variable concentrations of alcohol will, to some extent, affect the rate of absorption of the glucosides. I, therefore, consider it advisable to adjust all dilutions of the tincture to the same alcoholic strength.

Temperature control is a factor which should not be disregarded entirely. Obviously if the ouabain test is made at one temperature and the digitalis test is made at some other temperature, there will be, no doubt, a difference in the net result, but if the ouabain test is made in parallel, in the same water bath and at the same time, I see no

* Active collaborators.

reason why a variation in temperature of several degrees, either above or below twenty, should not be quite permissible.

The manner of performing the injection may have some bearing upon the final results. By injecting the solution through the muscle of the hind leg the chances are, because of the thickness of the muscle at this point, that there is less chance of error, due to leakage.

Frank B. Fisk, Pitman-Moore Co., Indianapolis.*—We had considerable difficulty with absorption. The end point taken was a blanched ventricle with markedly distended auricle capable of only slight stimulation at the 1 hour period.

I note that from the results obtained, a number of the laboratories would standardize high. If this high limit would be within the range of tolerance, their products should be very active therapeutically. The range of error appears considerable, yet I note fairly concordant results as to the relative strengths of the submitted tinctures by the individual operators, and the range of error on some of these operators is not out of reason.

I believe that further cooperative work might serve to establish in the various laboratories a factor of error which, when further proven, would enable all of us to put out a product of more nearly an equal strength. I further believe that work of this kind could be undertaken in the winter months to note whether there is any marked difference in the end points of these frogs as compared with that of your June assay. If more of the original tincture were on hand, it might also tend to give us something definite as regards the rate of deterioration.

These remarks are offered merely as suggestions for thought. It strikes me that from the biological method, this assay is comparatively cheap, readily performed, and enables us to give the physician a product that is active therapeutically. The observing physician is interested primarily in the therapeutic effect. If he uses a certain manufacturer's tincture and knows the dose required to produce results, he is very liable to continue using or specifying this tincture, whether the product be full strength or under strength. I know that we should always aim at a full therapeutic efficiency and one that has a high limit as well as a low limit.

*G. S. Gittinger, Pharmacological Laboratory, F. D. I. Administration.**—From the tabulated results of these assays it is seen that if more careful attention were paid to detail, as recommended in the U. S. P. X, there might be less discrepancy in results. Weight of frogs, temperature, and alcoholic concentration are important items to note, but care and accuracy are more important, also a sufficient number of frogs should be used to justify a report of a certain potency as recommended, and age and conditions of storage of the tinctures should be taken into consideration. As these tinctures were not assayed at the same period, some may have deteriorated from age and from carelessness in storage. The results are useful, and it is to be hoped that if repeated next year more consistent results may be obtained and more attention be given to detail.

Howard T. Graber, Chairman, Sub-Committee on Pharmacological Assay, American Drug Manufacturers Association.—I have just glanced over this report and have not had time to study it as thoroughly as I should like, but my first impression is that variations in reported strength are as great, if not greater, in this report than they were in the report which I presented to the American Drug Manufacturers Association.

Gilbert L. Harvey, Harvey-Pittenger Co., Philadelphia.*—We had considerable difficulty in obtaining satisfactory frogs. Those obtained weighed 5–20 grams. They were stored at 22°C. Results obtained were very unsatisfactory and not at all concordant. A new lot of frogs was received and stored at 23°–25°C. They were reduced with ice to 18°C. the night before an assay. In two months sample C showed a deterioration of 1/3 of its activity. I do not know whether you have taken into consideration the

* Active collaborators.

dates when these assays were carried out by the various collaborators but I feel this is an important factor.

*Chas. C. Haskell, Department of Pharmacology, Medical College of Virginia, Richmond.**—Confusion of various lots of frogs and improper behavior of my temperature regulator caused much difficulty in the assay of the preparations submitted. I hope that it will be possible to carry out a similar investigation this fall or winter when we are able to get better results with our limited facilities than we ever can in the warm weather.

F. W. Heyl and C. A. Pomeroy, The Upjohn Company, Kalamazoo.—In looking over the list of results we find that only 3 or 4 of the 21 collaborators have come close to the results and have placed the strengths in their proper relationship. This is a much smaller percentage of agreeing results than we would expect, but it does not destroy our confidence in the fundamental correctness of the method.

The maximum variation on the 1-hour frog method was found by the American Drug Manufacturers Association to be 30 per cent¹.

We greatly appreciate the opportunity of this kind of work for the purpose of feeling on solid ground in respect to the comparative results. In this laboratory we do not attempt to shave the dosage down closer than 10 per cent because we feel that the other possibilities of error are of that dimension.

*Horace A. Holaday, E. R. Squibb & Son, New Brunswick.**—The results are less consistent than are usually obtained in our frog assays. This is attributed to a larger deviation in response of the frogs used. In our opinion the final values obtained are not influenced by error of technic, with the possible exception of any change in activity involved in the evaporation and redilution of samples. This factor is reduced by the use of three separate evaporations for each sample. In a tabulation of minimum systolic doses for the tinctures and for the standard ouabain, we have followed our usual procedure of indicating the maximum dose which is judged to produce a typical standstill in definitely less than 50 per cent of the animals injected and the minimum dose which is judged to produce a typical standstill in definitely more than 50 per cent of the animals injected. In assays of this sort the data are too limited for exact mathematical treatment or calculation of probable error. We made comparisons on the basis of the minimum pharmacopeial ouabain standard.

Reid Hunt, Department of Pharmacology, Harvard Medical School, Boston.—The majority seem to have come reasonably near the relative strengths of the three preparations, but were rather far off in their comparisons with the U. S. P. standard.

Of course this is what gave Magnus and some others some difficulty and led them to prefer the use of a standard preparation of digitalis itself for comparison. The chief trouble with the Magnus method is that each worker has to make his own tincture of the standard leaf and this brings in a new source of error as well as trouble.

Glenn L. Jenkins, University of Maryland, College of Pharmacy, Baltimore.—I have spent some time trying to correlate various factors in your table of cooperative assays and have about concluded that the most striking detail is the lack of correlation. The course, weight, and temperature control do not seem to parallel in a sufficient number of instances to draw positive conclusions. Where many frogs have been used it does not appear to have increased the accuracy of the assay. In general, the percentage error is slightly higher than might be expected where so many frogs are used in the assay. If I were to hazard a guess in conclusion, I should say the experience of the assayist is probably the greatest factor to be considered.

A. S. Loevenhart, Department of Pharmacology and Toxicology, University of Wisconsin, Madison.—I am not at all surprised at the variation in the figures. Some of them are

¹ 1923 A. D. M. A. Report, p. 207.

* Active collaborators.

so far out of line that it is quite evident that an error has been made. I imagine that some of these people inject the digitalis into the dorsal or ventral lymph sac through the skin, which permits considerable leakage of the material. We always do our standardization by injecting into the anterior lymph sac, putting our needle through the mouth under the tongue. Under these conditions there is no leakage, and I think it is an important point in the standardization.

Carl Neilsen and H. C. Spruth, Abbott Laboratories, N. Chicago.*—Evaporation of alcohol and dilution of tincture were made to obtain fairly uniform final alcohol content of approximately 20 per cent. I do not believe that any one is experienced in bio-assay work of this sort unless he has been at it for several years.

H. W. Rhodehamel and C. C. Hargreaves, Eli Lilly & Co., Indianapolis.*—Each sample was evaporated under an air jet until all alcohol was driven off, taken up in 20 per cent alcohol, and made up to original volume. This in turn was diluted with distilled water for injection.

Allan Winter Rowe, Research Service, Evans Memorial Hospital, Boston.—I have gone over the digitalis data with a great deal of interest. The first thing that impresses me is the matter of your Illinois frogs. With nearly 1500 the average is poorer (83–105–88) than the Indiana group with one-third that number (79–115–94). Poor technic would seem to nullify any advantage to be derived from numbers (see 16). Whether the excellent results with the small group of New York frogs (7) is fortuitous you can tell better than I. If we regard the Illinois frogs as out of the picture the even temperatures would seem to be an advantage (2, 15°C. and 7, 18°C.). Note that the second Indiana group (9) varied from 20° to 25°C. and is the poorest of the three. True, the last group (21), which is the best, was at 20° but this is not so good as the New York group in the two higher portions.

Drawing conclusions from meager data—for only Illinois has a large representation—I should say:

1. Skilled operation is the primary requisite.
2. Indiana (or New York) frogs are to be preferred.
3. Underweight does not seem to be a serious source of error.
4. Groups should be large enough to absorb individual differences, say from 50 to 100 for each lot of tincture.
5. The lower temperatures would seem to offer some advantage. (There is a question in my mind, how closely these were controlled.)
6. Rather liberal limits of tolerance would seem to be necessary.

One other point occurs to me. The assayed values tend to run low. Is there chance for deterioration in individual lots through time, accidents of shipping, storage conditions, etc.?

E. W. Schwartze, Mellon Institute, University of Pittsburgh, Pittsburgh.—I must confess that results are not so good as one would hope for, although I am not surprised that there is a considerable number of discrepancies. It would seem that there is nothing left to do but to resubmit next year. The regrettable feature of these cooperative studies is that when the results are adverse, they do not necessarily speak against the method, although they are an index to what one could expect under similar circumstances.

F. A. Upsher Smith, Upsher Smith Co., Minneapolis.—I find myself unable to offer any remarks on the frog results that you sent me a copy of other than that they show a very wide variation as is to be expected in any physiological test whether based on frogs or cats.

Chas. E. Vanderkleed, Robert McNeil, Philadelphia.—I am greatly indebted to you for the copy of results obtained in the cooperative assay of tincture digitalis. Should you

* Active collaborators.

receive any further results, I will of course be very glad to receive a copy in due season. In the meantime this report will be of value to me in my association work.

The following active collaborators did not submit comments:

Norman McL. Harris and Fred W. Ward, Department of Health, Ottawa.

J. W. E. Harrison, 636 Race Street, Philadelphia.

Chas. F. Lanwermyer, John T. Milliken, St. Louis.

David I. Macht, Hynson, Westcott and Dunning, Baltimore.

Paul S. Pittenger, Sharp and Dohme, Baltimore.

L. W. Rowe, Parke-Davis and Co., Detroit.

Chas. A. Valloton, Meyer Brothers Drug Co., St. Louis.

DETERMINATION OF ATROPINE IN THE PRESENCE OF MORPHINE¹.

By L. E. WARREN (U. S. Food, Drug and Insecticide Administration, Washington, D. C.).

Tablets that purport to contain the salts of atropine, or scopolamine (hyoscine) in conjunction with the salts of morphine or occasionally with the salts of morphine and strychnine are usually listed as "Morphine and Atropine", "Hyoscine and Morphine", "Morphine, Atropine and Strychnine", "Scopolamine-Morphine Compound", etc. Ordinarily but two alkaloidal salts are present in the same tablet, but occasionally there are three. The proportion of morphine salts to the other therapeutic constituents is variable—usually ranging from about 20 to 1 to 75 to 1. Sometimes the total of the other potent constituents exceeds the morphine.

Since morphine has phenolic properties, it would seem that the quantitative separation from it of non-phenolic alkaloids such as atropine and scopolamine could be accomplished readily by employing the U. S. P. method for determining codeine sulfate in morphine sulfate. The morphine is fixed with an excess of sodium hydroxide solution and the liberated codeine is shaken out with chloroform². However, this method is inapplicable since most commercial morphine salts contain notable proportions of codeine salts as impurities. The atropine or other solanaceous alkaloid would be contaminated by the codeine in the morphine salt originally used in the tablet.

Suggestions have been made for the separation of atropine from strychnine³, codeine from morphine⁴, and atropine from pilocarpine and physostigmine⁵, but no satisfactory method has been available for the quanti-

¹ Read before the Division of Medicinal Products, American Chemical Society, Detroit, Mich., Sept. 7, 1927. Published through courtesy of *Industrial and Engineering Chemistry*.

² Reference to this procedure (using ether as the solvent) is made in *Methods of Analysis*, A. O. A. C., 1925, 397, but no account is taken of the codeine always present in morphine salts.

³ Fuller, *J. Ind. Eng. Chem.*, 1910, 2: 378.

⁴ U. S. P. X, 242.

⁵ Pohl, *Therap. Monatsh.*, 1910, 24: 691.

tative estimation of atropine in the presence of morphine with the exception of one involving the application of biological principles. Munch's method¹, which depends upon the determination of the threshold dose of atropine which will cause mydriasis in a young cat, gives results that are accurate within about 10 per cent. Morphine and its derivatives do not interfere.

It appeared feasible to separate the total alkaloids in the tablets into two fractions, one containing morphine only and the other the "total alkaloids not morphine", by the U. S. P. method for determining the codeine in morphine sulfate. The next steps were to destroy the atropine in the residue of "total alkaloids not morphine" by the Fuller method, determine the total of remaining alkaloids, and estimate the atropine by difference.

A mixture was prepared in the following proportions:

	gram
Atropine sulfate.....	1
Morphine sulfate.....	.25
Lactose.....	.74

Portions of the thoroughly mixed ingredients were assayed as follows:

Weigh, accurately, about 4 grams of the mixture, corresponding to about 1 gram of morphine sulfate and about 0.040 gram of atropine sulfate; dissolve in 20 cc. of warm water; transfer the solution to a separator; and wash the container with two portions of 5 cc. each of sodium hydroxide test solution, adding each portion to the separator. Shake the solution with four successive portions of 15, 10, 10, and 10 cc. of chloroform. Wash the combined chloroform solutions with 5 cc. of water and filter the solvent through a pledget of cotton placed in the stem of the separator into a tared Erlenmeyer flask. Wash the aqueous layer with 5 cc. of fresh chloroform, draw off the chloroform layer into the tared flask, recover most of the chloroform by distillation, add 2 cc. of absolute alcohol², and evaporate the solution on a steam bath while rotating the flask continuously. Dry the residue at 80°C. and weigh the residue as "total alkaloids not morphine".

Add 10 cc. of approximately 0.5 *N* alcoholic potassium hydroxide to this residue; transfer the mixture to a pressure flask, washing the original container with five portions of 2 cc. each of alcohol; stopper the flask securely; and heat it on the steam bath for 1 hour. Cool the solution and pour the alkaline solution into an evaporating dish, wash the bottle with 10 cc. of alcohol in small portions, follow by 10 cc. of water, and evaporate the resultant solution until the alcohol has been dissipated. Transfer the residue to a separator and wash the dish with 10 cc. of water, in small portions. Shake the solution with three portions of 10 cc. each of chloroform or more to insure complete extraction, wash the combined chloroform extracts with 5 cc. of water, and discard the wash water. Shake the chloroform extracts with three portions of 10 cc. each of 1 per cent sulfuric acid. Add just sufficient sodium hydroxide test solution to the combined acid solutions to render alkaline, shake the mixture with 3 portions of 10 cc. each of chloroform—or more if extraction is not complete—wash the combined chloroform extracts with 5 cc. of water, discard the wash water, evaporate the solvent almost to

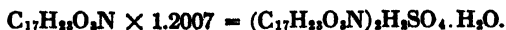
¹ *This Journal*, 1927, 10: 383.

² In the earlier tests this addition of alcohol was not made. In the later tests this was done in conformity with the procedure.

TABLE 1.
Results of analyses of atropine-morphine mixtures.

SAM- PLE	CALCULATED WEIGHT OF ATROPINE TAKEN	WEIGHT OF TOTAL ALKALOIDS NOT MORPHINE	WEIGHT OF CODEINE FOUND	WEIGHT OF ATROPINE BY DIFFERENCE	THEORY FOR ATROPINE (BY WEIGHT)	TOTAL 0.02 N ACID CONSUMED	0.02 N ACID CONSUMED BY CODEINE	0.02 N ACID CONSUMED BY ATROPINE	WEIGHT OF ATROPINE CALCULATED FROM TITRATION	THEORY FOR ATROPINE (BY TITRATION)
	gram	gram	gram	gram	per cent				gram	per cent
A	0.03362	0.0382	0.0117	0.0265	78.8					
B	0.03322	0.0406	0.0115	0.0291	87.6					
C	0.0332	0.0411	0.0114	0.0297	89.5					
D	0.0340	0.0425	0.0096	0.0329	96.8					
E	0.0275	0.0387	0.0082	0.0305	110.9					
F	0.03368	0.0439	0.0077	0.0362	107.5	7.023	1.25	5.77	0.03340	99.2
G	0.03338	0.0415	0.0072	0.0343	102.8	6.77	1.14	5.63	0.03258	97.6
H	0.03332	0.0491	0.0079	0.0412	123.6	6.974	1.145	5.83	0.03373	101.2
I	0.03332					6.853	1.402	5.45	0.03154	94.7

dryness on the steam bath, dissipate the last of the solvent by cautious rotation of the container, dry the residue at 80°C., and weigh as codeine. Subtract the weight of codeine found from the "total alkaloids not morphine". The remainder is considered as atropine.



Repeat the determination but instead of weighing the "total alkaloids not morphine" dissolve the residue in 10 cc. of 0.02 *N* sulfuric acid and titrate back with 0.02 *N* sodium hydroxide, using methyl red as indicator. Record the quantity of 0.02 *N* sulfuric acid consumed. Make the solution alkaline with ammonia water, shake out with chloroform in the usual way, evaporate the solvent, and destroy the atropine in the residue by the method given above. Recover the undecomposed alkaloid as described in the second paragraph, titrate the residue supposed to be codeine, subtract the amount of 0.02 *N* sulfuric acid consumed by the codeine from that consumed by the "total alkaloids not morphine" and calculate the difference to atropine sulfate.

Each cc. of 0.02 *N* sulfuric acid = 0.005786 gram of atropine or 0.006947 gram of atropine sulfate, U. S. P.

In some of the trials the "total alkaloids not morphine" were weighed and afterward titrated. As a general rule the findings for atropine obtained by weight were higher than those by titration.

The findings obtained in several trials are given in Table 1.

The method was also tried when a Type C mechanical extractor¹ was used for the extractions from alkaline solution into chloroform, a separator being employed for such extractions as were conducted in the opposite direction. The method was found to be impracticable, however, as some morphine invariably came over when the extraction was prolonged.

Specimens of the material were sent to several pharmaceutical manufacturers, schools of pharmacy, and stations of the (then) Bureau of Chemistry, with the request that they be assayed by the methods submitted. Reports were received from the following collaborators:

- E. O. Eaton, San Francisco;
- H. O. Moraw, Chicago;
- C. K. Glycart, Chicago;
- R. L. Horst, New Orleans;
- Louis Fischer and Leon Richards, College of Pharmacy, University of Washington, Seattle;
- A. H. Clark, School of Pharmacy, University of Illinois, Chicago;
- F. W. Heyl, The Upjohn Co., Kalamazoo; and
- Parke, Davis & Co., Detroit.

The collaborative results are shown in Table 2.

In addition, F. W. Heyl of The Upjohn Co. submitted a report, but it was not in quantitative terms. He stated that in control assays checks could not be obtained.

¹ Palkin, Murray and Watkins. *Ind. Eng. Chem.*, 1925, 17: 612.

TABLE 2.

Results of analyses of atropine-morphine mixtures by collaborators.

COLLABORATORS	E. O. E.	H. O. M.	C. K. G.	R. L. H.	L. V.	L. R.	P. D. & CO.*	A. H. C.†
Gravimetric	99	107	81	172	136	166	89.4	89.7
Recovery	96			179	96	56	76.5	90.0
(per cent)					129	164	93.0	80.7
						140	92.8	
							96.6	
Volumetric	93	91	99	90	12.8	Practi-	89.4	
Recovery	93					cally	94.4	
(per cent)						nothing	91.2	
							92.0	
							94.4	
							94.6	

* Mixture of morphine hydrochloride, atropine sulfate, and lactose, containing 1% atropine sulfate.

† Specimen prepared by analyst, but composition was the same as others.

The writer also made a few experiments to determine whether atropine is completely destroyed by the saponification process, as outlined in the methods given. In two tests, 0.1 gram each of atropine sulfate was saponified with 20 cc. of 0.5 *N* alcoholic potassium hydroxide, and after evaporation of the alcohol the unchanged bases were removed by shaking with chloroform. The chloroform extract was washed with water, and the bases were removed by several shakings with 1 per cent sulfuric acid. The bulked acid solution was made alkaline with ammonia water, and the bases were removed by chloroform and titrated in the usual way. The recoveries were 0.0031 gram and 0.0013 gram, respectively, calculated as atropine sulfate corresponding respectively to 96.9 and 98.7 per cent destruction. The recovered bases had no mydriatic properties when tested on cats. It is thus seen that the noticeable error introduced by the imperfect saponification of the atropine will be reflected in a correspondingly large yield of codeine with a parallel loss in the atropine estimation.

CONCLUSIONS.

The results show that experienced analysts familiar with the technic can obtain results within about 10 per cent of the truth, while inexperienced analysts are apt to fail. The method is long and time-consuming. In its present state, therefore, it cannot be recommended except as an occasional control in tablet production.

The writer wishes to acknowledge his appreciation to the several pharmaceutical manufacturers and to others who have aided in this study.

CONTRIBUTED PAPERS.

THE MOISTURE CONTENT OF OLEOMARGARINE.

By ROBERT H. KERR (Meat Inspection Laboratory, Bureau of Animal Industry, Washington, D. C.).

Although oleomargarine is produced and consumed in large quantities, comparatively little published information is available regarding the chemical composition of the product as it is found on the market. The analyses that have been published are mostly those of individual samples, and they apply to single brands or to the products of individual factories. The series of determinations presented in this paper, in which samples of all brands of oleomargarine prepared at establishments operating under Federal meat inspection were examined for moisture within a comparatively short period of time, affords a comprehensive view of the moisture content of oleomargarine as it is now produced in the United States.

ORIGIN AND CHARACTER OF SAMPLES.

Collection of the samples, incident to the enforcement of the Act of Congress of June 30, 1906, known as the meat inspection act, was limited to establishments at which inspection is maintained under authority of that act. This field includes all establishments that manufacture oleomargarine consisting in part of fats derived from the carcasses of cattle, sheep, swine, or goats, but it does not include establishments the product of which consists exclusively of a mixture of vegetable fats churned with skimmed milk, milk, or cream. Samples were collected from 37 manufacturing plants located in 18 cities distributed throughout the United States by inspectors assigned to these establishments. The analyses were made in the 7 meat-inspection laboratories of the Bureau of Animal Industry located, respectively, at Chicago, Ill.; Kansas City, Kans.; New York, N. Y.; Omaha, Nebr.; San Francisco, Calif.; St. Louis, Mo.; and Washington, D. C.

The samples were found to belong to two principal classes, (1) oleomargarine intended to be used generally as a substitute for butter, and (2) oleomargarine intended primarily or exclusively for use in cooking or baking. No attempt was made to separate or grade either class according to quality. There are also reported 16 analyses of oleomargarine consisting of vegetable fats churned with skimmed milk and containing no fats derived from the carcasses of meat animals. These 16 samples represent a product that is not subject to the meat-inspection act and is not marketed under the stamp of Federal meat inspection, although they were prepared at establishments at which Federal meat inspection is maintained.

TABLE 1.

Results of analyses of 159 samples of oleomargarine intended for use as a substitute for butter.

SAMPLE NO.	WATER	MILK SOLIDS AND SALT	FAT	SAMPLE NO.	WATER	MILK SOLIDS AND SALT	FAT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
2,248	10.0	1,931	13.53
2,249	11.5	6.2	82.3	1,932	12.56
2,250	13.0	2.8	84.2	1,935	13.89
2,251	11.8	1.2	87.0	3,001	14.33
2,252	8.0	3,004	10.65
2,253	7.1	3,274	8.93
2,254	7.0	3,275	10.62
2,267	13.5	5.8	80.7	3,276	9.81
2,268	13.1	6.1	80.8	3,277	9.63
2,387	9.1	3,279	9.96
2,390	7.7	3,280	8.98
2,391	7.5	3,269	11.60
2,392	11.0	3,270	11.42
2,393	8.1	3,271	9.58
2,394	9.7	3,272	9.61
2,395	10.0	3,265	11.50
2,429	11.5	3,266	12.60
2,430	13.0	4.7	82.3	3,267	9.88
2,431	9.5	3,268	8.58
2,433	8.5	3,484	9.91
2,434	11.0	3,485	10.63
2,435	14.0	4.2	81.8	3,486	14.18
2,436	9.5	3,487	10.31
2,437	10.2	3,126	9.50
2,438	9.2	3,127	8.82
2,439	8.8	3,128	8.11
2,455	11.2	3,129	10.76
2,456	12.7	3,130	10.76
2,457	11.5	3,131	7.38
2,469	8.5	3,132	8.32
2,470	11.5	3,133	10.78
2,471	9.3	29	9.46
2,472	10.5	31	9.64
2,473	10.0	32	10.28
2,474	10.5	33	11.73
2,475	10.0	34	9.45
2,478	5.0	35	8.12
2,572	7.9	36	10.00
2,575	6.5	37	10.81
2,576	6.0	321	16.0
2,577	10.9	322	11.2
2,578	7.2	323	11.6
2,579	10.1	324	14.1
179	12.2	326	12.1
180	8.0	327	15.3
2,972	13.0	328	15.9
201	14.37	329	11.3
202	6.20	3,211	14.1
203	9.85	3,212	10.3
204	11.55	352	9.3
205	10.3	353	13.4
23	12.99	3,382	9.64
24	10.95	3,387	11.28
25	8.92	3,388	11.30
26	13.09				

TABLE 1.—Continued.

Results of analyses of 159 samples of oleomargarine intended for use as a substitute for butter.

SAMPLE NO.	WATER	MILK SOLIDS AND SALT	FAT	SAMPLE NO.	WATER	MILK SOLIDS AND SALT	FAT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
3,380	11.92	3,748	14.04
3,429	11.34	226	11.07
3,430	10.71	206	7.7
3,431	8.34	207	12.2
3,432	10.27	263	11.0
3,459	8.55	258	10.8
3,460	9.18	260	11.2
3,461	11.35	261	12.0
3,462	12.92	262	10.7
3,463	9.34	871	11.4
3,464	8.46	5	11.9
3,465	9.05	408	6.94
3,551	11.61	971	14.34
3,552	10.37	97	14.98	4.16	80.86
3,553	11.86	98	14.74	3.91	81.35
3,554	12.35	430	13.3
3,555	10.91	429	13.4
3,556	11.09	878	13.58
3,557	12.11	965	13.72
3,558	10.28	2	11.56
3,721	14.46	581	12.0
3,722	9.38	402	10.88
3,723	11.62	582	13.2
3,724	12.78	880	10.22
3,747	10.63	881	12.14

All samples were prepared for analysis by the official method¹; moisture was determined by the official method¹, and fat was determined by the indirect official method¹.

Oleomargarine Intended to be Used Generally as a Substitute for Butter.

There were examined 159 samples of oleomargarine intended to be used generally as a substitute for butter. These samples, which were produced in 37 different establishments located in 18 cities, represent 100 separate brands or formulas. The number of samples exceeds that of brands or formulas because in many cases the same brand was prepared in two or more establishments operating under the same management. Samples of one widely distributed brand were collected from 9 different establishments. During the period covered by the tests samples from 3 establishments were found not to be in conformity with the meat inspection regulations and were made the subject of corrective action. They are not included in this report, although analyses of the brands represented by them are included. The results of moisture determination, together with the results of determination of fat in those cases in which fat was determined, are shown in Table 1.

¹ *Methods of Analysis*, A. O. A. C., 1925, 276.

Oleomargarine Intended for Use in Cooking and Baking.

There were examined 45 samples of oleomargarine intended to be used for cooking and baking and not intended to be used generally as substitutes for butter. These samples represent the product of 24 establishments located in 14 cities and include 39 brands or formulas. The results are given in Table 2.

TABLE 2.

Results of analyses of 45 samples of oleomargarine intended for use in cooking and baking.

SAMPLE NO.	WATER	MILK SOLIDS AND SALT	FAT	SAMPLE NO.	WATER	MILK SOLIDS AND SALT	FAT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
2,269	6.2	3,134	4.26
2,388	6.5	38	13.73
2,389	4.5	39	6.13
2,440	8.0	324	8.4
2,441	11.0	3,211	10.2
2,442	5.5	3,213	13.7
2,443	12.8	350	5.6
2,476	3.7	351	14.9
2,477	4.8	3,384	4.60
2,479	11.00	3,433	5.89
2,480	5.00	3,434	13.19
2,481	14.0	2.3	83.7	222	5.9
206	11.64	223	5.16
207	10.54	227	11.29
208	1.08	228	6.5
27	0.15	229	4.7
28	9.37	230	11.1
1,933	8.99	204	5.08
1,934	7.16	205	14.3
3,003	6.57	409	6.47
3,278	6.83	583	12.5
3,273	6.14	410	12.96
3,264	6.73				

Oleomargarine Consisting of Vegetable Fats Churned with Skimmed Milk.

There were also examined 16 samples of oleomargarine consisting of vegetable fats churned with skimmed milk and containing no fats derived from the carcasses of meat animals. These samples, which were collected from 10 establishments located in 5 cities, represent 15 different formulas or brands. The results are given in Table 3.

The results of the moisture determinations made on samples of this type would appear to indicate that the so-called "vegetable" or "nut" oleomargarine is somewhat more uniform in its moisture content than the oleomargarine made in part from the body fats of meat animals. The number of samples, however, is too small to justify definite conclusions on this point.

SUMMARY.

Samples of the oleomargarine produced by 37 establishments located in 18 cities distributed throughout the United States were collected, and

TABLE 3.

Results of analyses of 16 samples of oleomargarine consisting of vegetable fats churned with skimmed milk.

SAMPLE NO.	WATER
	<i>per cent</i>
209	11.36
210	10.58
211	12.56
21	14.28
22	12.37
310	12.97
311	13.63
1,936	14.47
1,937	12.51
3,002	7.03
221	13.8
224	10.3
225	11.4
231	12.63
189	10.6
259	9.7

the moisture content was determined. Results of examination of 159 samples representing 100 different brands show the moisture content of most of them to be above 8 per cent and below 14 per cent. Examination of 45 samples representing 39 brands of oleomargarine intended to be used in cooking and baking indicates that the moisture content of this product is generally less than that of oleomargarine intended for general use as a butter substitute. Examination of 16 samples representing 15 brands of oleomargarine consisting of vegetable fats churned with skimmed milk shows a moisture content similar to that observed in oleomargarine consisting in part of the body fats of meat animals and intended for general use as butter substitutes.

DETERMINATION OF ALCOHOLIC EXTRACTIVE IN GUM BENZOIN.

By T. N. BENNETT and C. F. BICKFORD (U. S. Food, Drug and Insecticide Administration, New York, N. Y.).

The method given in United States Pharmacopeia X for the determination of alcohol-soluble extractive of gum benzoin is apparently unsatisfactory for the reason that the extractive material is dried at a temperature of 110°C., at which temperature certain constituents of the extracted material are volatile.

Gum benzoin contains a number of substances which are soluble in alcohol and somewhat volatile at 110°C.¹ The chief of these substances,

¹ Wiener. Die Rohstoffe des Pflanzenreiches, 3 Aufl., I Band, S 408-17.

benzoic acid, would be lost in varying quantities on drying. To prevent this loss of volatile acid methods were devised depending on the neutralization of the extractive prior to drying, and the correction of the results was made accordingly. Methods were also tried involving the determination of alcohol-insoluble material, calculating the alcohol-soluble material by difference, and making suitable corrections for moisture content.

The following methods were compared, seven samples of gum benzoïn being used:

Method 1 (U. S. P.).—Macerate about 2 grams of the prepared drug (paragraph VI), accurately weighed, in about 70 cc. of alcohol in a suitable flask. Shake during 8 hours at 30 minute intervals and allow to stand 16 hours without shaking. Filter and wash the flask and residue with small portions of alcohol until the filtrate measures 100 cc. Evaporate a 50 cc. aliquot portion to dryness in a suitable tared dish on a water bath and dry to constant weight at 110°C. Calculate the percentage of anhydrous extractive from the weight of drug taken.

Method 2.—Macerate about 2 grams of the prepared drug (paragraph VI), accurately weighed, in about 70 cc. of alcohol in a suitable flask. Shake during 8 hours at 30 minute intervals and allow to stand for 16 hours without shaking. Filter and wash the flask and residue with small portions of alcohol until the filtrate measures 100 cc. Add to a 50 cc. aliquot portion an equal volume of distilled water and 1 cc. of phenolphthalein solution (1 gram per 100 cc.). Titrate slowly with 0.1 N sodium hydroxide solution to a permanent pink end point. Evaporate and dry the residue to constant weight at 110°C. Correct the residue for the quantity of phenolphthalein added and for the quantity of sodium added and hydrogen lost.

Method 3.—Follow the method outlined in the U. S. P., using a tared filter paper, weighing the residue, and calculating alcohol-soluble extractives by difference. Correct for water as determined by distillation with xylol.

Method 4.—Treat as in Method 3, except to make the filtration on a tared Gooch crucible prepared with a thin asbestos pad, and wash the residue with alcohol, using a gentle suction if necessary, until the residue is entirely free from alcohol-extractive matter. Determine this point by treating successive small portions of the filtrate with water and continuing the washing until no cloudiness is observed. Check the complete absence of extractive matter by evaporating another successive portion of the filtrate to dryness on a watch glass to observe the absence of residue.

Method 5.—Macerate about 2 grams of the prepared drug accurately weighed in about 70 cc. of alcohol, using a 100 cc. volumetric flask. Shake during 8 hours at 30 minute intervals and allow to stand for 16 hours without shaking. Make up to the mark with alcohol, shake, allow to settle somewhat, and filter through a dry paper. Pipet 50 cc. of the filtrate and treat as in Method 2, beginning "add to a 50 cc. aliquot".

Method 6.—Weigh 2 grams of the sample into a dried and tared paper extraction thimble, using a glass-stoppered weighing bottle as a container. Extract in a continuous extraction apparatus with 95 per cent alcohol containing about 0.5 gram of sodium hydroxide for 5 hours. Dry and weigh thimble and calculate alcohol extractive matter plus water by difference. Deduct water as determined by the xylol distillation method from the result and report as alcoholic extract.

The following results were obtained:

SAMPLE NO.	NY 7203	NY 7203	NY 7550	NY 7550	NY 7704	NY 8137	NY 8141
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Method 1.....	77.25 76.88	69.70 68.40	68.90 70.15	67.68	85.28	69.76 68.25	67.85 65.57
Average.....	76.88	69.05	69.52	67.68	85.28	69.00	66.11
Method 2.....	79.49 79.46	71.00 69.11	75.89 73.03	70.03	86.07	71.25	69.60
Average.....	79.48	70.06	74.46	70.03	86.07	71.25	69.60
Method 3.....	79.25 79.82	71.52 71.48	72.52 72.52	69.20 69.61	86.04 86.48	75.22	72.52 72.13
Average.....	79.54	71.50	72.52	69.41	86.26	75.22	72.32
Method 4.....	79.89 80.16	71.68 72.28	73.73 72.68	69.92	87.11	76.17 75.57	72.28 72.06
Average.....	80.02	71.98	73.20	69.92	87.11	75.87	72.17
Method 5.....	80.29 82.30	71.98 73.50	76.84 76.83	72.08 ...	89.74 ...	73.40	69.90 ...
Average.....	81.29	72.74	76.83	72.08	89.74	73.40	69.90
Method 6.....	81.53 81.61	73.50 73.46	74.35 74.59	72.35 72.36	88.13 87.79	76.80 76.96	74.02 73.43
Average.....	81.57	73.48	73.47	72.36	87.96	76.88	73.72
Water (xylol).....	2.60	3.45	3.35	3.70	2.40	4.60	4.60

The method outlined in U. S. P. X is long and tedious and yields results which are obviously in error, since several substances which might be considered as active ingredients of gum benzoin are lost during the course of the determination. Method 2, involving the neutralization of the alcoholic extract prior to evaporation, gives somewhat better results, but it is also rather long and tedious. Methods 3, 4, and 6 depend on the determination of alcoholic extractive by difference; they yield comparable results, but Method 6 is easier of manipulation and probably represents more nearly the actual quantity of alcohol-extractive material in the sample. Method 5 is open to the same objection as Method 2, in that it is somewhat tedious and involves an error in not taking account of the volume of insoluble material in the sample. Method 6 is quite simple and gives results in the shortest time.

STUDIES OF TOMATO QUALITY. II EFFECT OF SOIL MOISTURE UPON THE PERCENTAGE OF DRY MATTER IN THE FRUIT.

By R. E. BROOKS and JOHN H. MACGILLIVRAY¹ (Agricultural Experiment Station, Lafayette, Ind.).

The variability of tomato fruits in moisture content is of interest for several reasons. (1) The water content greatly affects the yield of pulp that can be obtained per ton of raw stock. At the present time the canner is purchasing this material without any regard to the quantity of finished product that can be manufactured from each ton of fruit. (2) The industry would suffer fewer depressions if tomatoes were purchased on the basis of dry matter, the valuable portion of the fruit. (3) The detection of added water to canned tomatoes necessitates a knowledge of the variation in water content due to environment. Bigelow², in field experiments, was unable to find any definite correlation between rainfall and water content of tomatoes.

SPECIFIC GRAVITY OF JUICE FROM FIELD-GROWN TOMATOES.

The Tomato Products Company, Paoli, Indiana, kindly furnished the data for Table 1. The specific gravity of the raw cyclone pulp was available for 6 years, and the readings were made on an Abbé refractometer. The rainfall records were obtained from a Government Station located at Paoli. Water was used in the factory operations to cleanse the fruits, and all fruits received a similar treatment. Tomatoes grown in a large area for one factory, however, would undoubtedly exhibit daily variations in specific gravity because of fluctuations in disease, fertility, and rainfall. These data are included to prove that the water content of tomatoes varies during the same season and from year to year. Fig. 1 shows some correlation between the rainfall and the moisture content of the fruits. In order to obtain definite information on the effect of soil moisture on the dry-matter content of the fruit, results from two experiments were obtained under conditions of controlled soil moisture in the greenhouse.

PLANTS GROWN IN POTS AT 30, 50, AND 70 PER CENT SOIL-MOISTURE CONTENT.

Baltimore tomato plants were maintained under the different moisture conditions as accurately as possible by weighing at least every other day. Ten plants for each treatment, or a total of sixty plants for the two experiments, were planted in rich greenhouse compost soil when the first

¹ Contribution from the Department of Horticulture, Purdue University, published with the approval of the Dean of the School of Agriculture and the Director of the Experiment Station. Part I was published in *Proc. Am. Soc. Hort. Sci.*, 23, pp. 208-15.

² *This Journal*, 1919. 3: 1.

Variation in Rainfall and Water Content of Cyclone Pulp for Six Years.

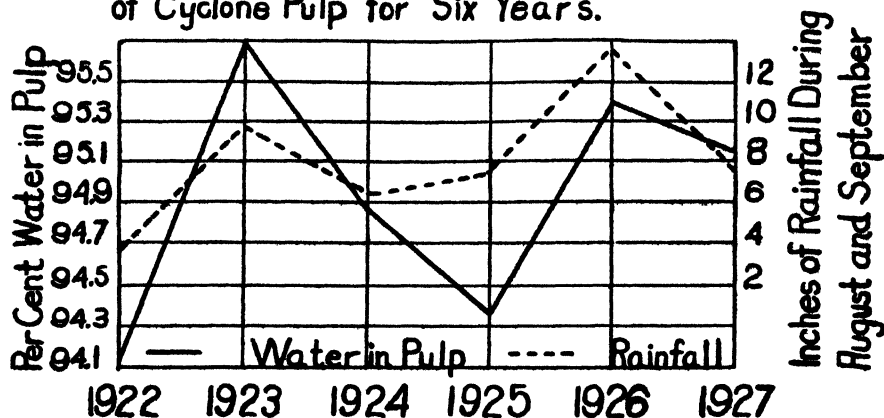


Figure I

blossom clusters began to open. In order to determine the water-holding capacity of the soil, soil tubes were filled, compacted, and weighed; they were then set in water until saturated and reweighed. The weight obtained was corrected for the water the soil previously contained by drying the samples at 100°C. All flowers were hand pollinated.

TABLE 1.

Data on the specific gravity of raw cyclone pulp during different seasons.

	1922	1923	1924	1925	1926	1927
Sp. gr. of raw pulp (average) .	1.0249	1.0185	1.0218	1.0239	1.0197	1.0207
Rainfall, August and September (inches)	3.74	9.85	6.33	7.47	13.49*	7.38
Rainfall, July, August, September (inches)	8.06	13.87	7.46	9.58	16.98*	10.60
Daily difference in sp. gr. (average)	0.0038	0.0027	0.0032	0.0036	0.0033	0.0035
Sp. gr. first third of season (average)	1.0256	1.0206	1.0225	1.0252	1.0201	1.0213
Sp. gr. second third of season (average)	1.0253	1.0191	1.0212	1.0250	1.0200	1.0209
Sp. gr. last third of season (average)	1.0240	1.0164	1.0215	1.0216	1.0191	1.0197
Smallest sp. gr. of the season .	1.0144	1.0134	1.0183	1.0180	1.0162	1.0161
Highest sp. gr. of the season .	1.0286	1.0243	1.0264	1.0312	1.0254	1.0279
Number of sp. gr. determinations	405	249	112	313	200	180

* August 1, 5.4 inches of rainfall.

The records of number, weight, and volume of fruit are shown in Table 3. As the soil moisture increases, these various values also increase.

RELATION OF SPECIFIC GRAVITY AND DRY-MATTER CONTENT OF THE FRUIT TO SOIL-MOISTURE CONTENT.

The specific gravity of the ripe fruit was obtained by weighing it in the air (W_a) and then in alcohol (W_b) and using the following formula for determining specific gravity:

$$\frac{W_a}{W_a - W_b} \times \text{specific gravity of alcohol} = \text{specific gravity of fruit.}$$

$$\frac{W_a - W_b}{\text{Sp. gr. of alcohol}} = \text{volume of fruit}^1.$$

The volume was calculated from a similar formula.

The tomatoes were prepared for sampling by grinding in a Nixtamal mill. Duplicate 10 gram samples were dried in a vacuum oven for 10 hours at 70°C. The drying was done at a pressure not greater than 100 mm. of mercury for the last 6 hours. To obtain the specific gravity and percentage of dry matter, 128 ripe tomatoes were used.

The results in Table 3 indicate that the dry matter content of the fruit is in inverse proportion to the water content of the soil. The specific gravity of the fruit seems to increase with the increase of the percentage of dry matter, and readings of individual fruits given in Table 2 indicate that this determination is not an accurate method of measuring the quantity of dry matter in the fruit. The presence of gases and the varying percentages of seeds and skins are probably responsible for the variation in the specific gravity readings.

To determine whether or not the presence of seeds and skins might influence the relative percentages of dry matter, these parts were removed from all the fruits used in Experiment II. It was found, however, that no change occurred (Table 2).

Buying or selling tomatoes on a weight or volume basis disregards the food value of the product. It is similar to the purchase of milk without the aid of a Babcock test and consequently with no regard for the fat content of the milk. In manufacturing tomato pulp, the water content of the fruit is one of the factors that greatly influences the yield obtained per ton of raw stock. If tomatoes of a high moisture content break into pieces in the can to a greater extent than those of low moisture content, as is commonly believed, such a canned product brings a lower price because the high percentage of soil moisture affects the value of the fruit. Fancy canned tomatoes consist usually of whole fruits. Excessive rainfall during the two weeks preceding ripening will have a most harmful effect on the food value of tomatoes.

¹ In the first paper of this series this formula was incorrectly printed.

TABLE 2.

Individual plant and fruit records giving the variability in dry matter of tomatoes grown in different soil moistures.

30% SOIL MOISTURE.				
NUMBER OF PLANT	NUMBER OF FRUIT	DATE OF RIPENING	SPECIFIC GRAVITY OF FRUIT	DRY MATTER IN FRUIT
1	a	10/15	1.0101	<i>per cent</i> 11.60
	b	10/29	1.0122	12.91
3	a	10/8	0.9945	9.78
	b	10/15	1.0097	9.76
8	a	10/22	1.0100	11.54
	b	10/22	1.0100	10.50
	c	10/22	1.0250	11.53
70% SOIL MOISTURE.				
21	a	10/21	0.9995	7.69
	b	10/21	1.0065	7.59
	c	10/23	0.9983	7.78
	d	10/23	0.9951	7.73
22	a	10/15	0.9962	8.57
	b	10/21	0.9780	8.40
	c	10/23	0.9902	8.17
	d	10/29	0.9993	8.33
23	a	10/8	0.9912	7.57
	b	10/21	1.0032	7.67
	c	10/21	0.9967	7.42
	d	10/23	0.9931	7.13
	e	10/29	1.0030	7.18
	f	10/29	1.0050	6.87

Some plants produce fruits of a higher percentage of dry matter than others even though they are grown under the same soil-moisture conditions (Table 2). The plants grown in soil at 70 per cent moisture produced fruits that had a uniform percentage of dry matter. The instability of tomato fruits in regard to their percentage of dry matter is shown by the following data of maximum value at 30 per cent soil moisture and the minimum value at 70 per cent soil moisture. In experiment I the highest percentage of dry matter at 30 per cent soil moisture was 12.95, and the lowest percentage of dry matter at 70 per cent soil moisture was 6.87. In experiment II, under similar conditions, the values were 10.88 per cent dry matter for 30 per cent soil moisture and 5.13 per cent dry matter for 70 per cent soil moisture.

TABLE 3.
Summary of data on tomato fruits.

DETERMINATIONS	EXPERIMENT I WHOLE FRUITS			EXPERIMENT II FRUITS MINUS SEED AND SKIN		
	30% Soil Moisture	50% Soil Moisture	70% Soil Moisture	30% Soil Moisture	50% Soil Moisture	70% Soil Moisture
Average number of fruits per plant	1.72	2.66	4.57	4.3	6.0	5.5
Average weight of fruit per plant (grams)	58.07	182.77	485.71	378.75	712.05	737.41
Average weight of each fruit (grams)	39.0 ± 1.983	70.0 ± 3.137	104.0 ± 3.814	88.0 ± 4.524	121.8 ± 4.443	125.0 ± 4.192
Average volume of each fruit (cc.)	37.3 ± 1.983	69.5 ± 3.167	102.5 ± 3.850	89.3 ± 4.354	125.0 ± 4.314	130.0 ± 4.253
Specific gravity of fruit	1.0108 ± .0015	1.0066 ± .0011	.9960 ± .0008	.9982 ± .0016	.9738 ± .0040	.9660 ± .0041
Dry matter in fruit (per cent)	10.93 ± .172	9.74 ± .077	7.80 ± .068	9.04 ± .093	7.07 ± .070	6.60 ± .074

SUMMARY.

1. The percentage of dry matter of a tomato fruit varies inversely with the percentage of soil moisture.
2. Seventy per cent soil moisture produced the largest quantity, the largest number, and the largest sized fruit.
3. It seems that the percentage of dry matter varies with the percentage of soil moisture.
4. The percentage of dry matter in tomatoes greatly affects the cost per unit of food purchased on a weight or volume basis.

AN ELECTRICALLY HEATED FURNACE FOR ORGANIC COMBUSTIONS¹.

By MAX PHILLIPS and R. HELLBACH (Bureau of Chemistry and Soils,
U. S. Department of Agriculture, Washington, D. C.).

The ordinary electric combustion furnace of the three-unit type, such as is supplied commercially, has a number of disadvantages. In the first place, the resistance wire is so wound that the heating units become hotter in the center than at the ends, and the combustion tube is thus unequally heated. Furthermore, no provision is made for preheating the oxygen or for careful temperature control of the front end of the tube when lead

¹ 146th contribution from the Color and Farm Waste Division. This apparatus was demonstrated at the annual meeting of the Association of Official Agricultural Chemists, Oct. 31, 1927.

peroxide is used in the analysis of nitrogen-containing compounds. The same unit that heats the oxidized copper-gauze coil, which serves as an "oxidation buffer", is also used for burning the substance. When this unit is moved over the portion of the tube containing the boat, the oxidized copper-gauze coil is cooled, and its effectiveness as an "oxidation buffer" is greatly impaired.

The writers have constructed an electrically heated combustion furnace that has none of the disadvantages enumerated. The entire apparatus, including preheater, bubble-counter, and drying tube, is mounted on a board and thus makes one single and readily portable unit. As all the heating units are small, it is possible to use a smaller combustion tube and to make carbon and hydrogen determinations on 70–100 mg. samples, if so desired.

The construction of this furnace is shown in Fig. 1. Fig. 2 shows it assembled and ready for operation.

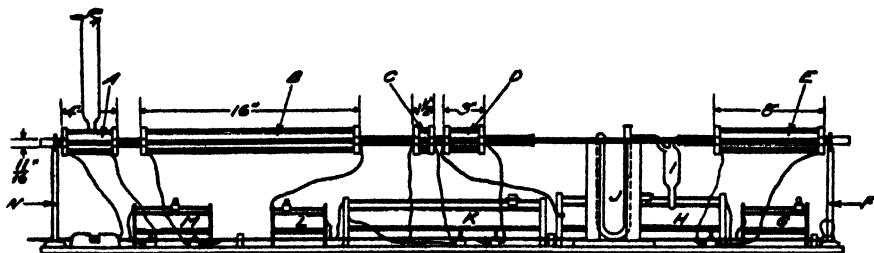


FIG. 1.

The heating units *B*, *C*, *D*, and *E* were constructed as follows: A copper tube having an outside diameter of $\frac{3}{4}$ inch and an inside diameter of $\frac{1}{4}$ inch was covered with mica and wound with No. 22 asbestos-covered nichrome wire. For *E*, 28 feet (total resistance 26 ohms) of wire, wound close together, was used. For *D*, *C*, and *B*, 13 feet (12 ohms), 8 feet (7 ohms), and 28 feet (26 ohms), respectively, of the No. 22 asbestos-covered nichrome wire was used. This wire was evenly wound around the tubes and then covered with a thick layer of alundum cement. This was wound with asbestos paper to a thickness of about 2 inches. It was then put in a 2 inch sheet-iron tube, and the ends were cemented with crushed pipe covering to prevent the leads from short-circuiting. Two $\frac{1}{8}$ inch holes were placed 5 inches from each end of *B* in order that the temperature of the combustion tube might be observed.

Heating unit *A* kept the lead peroxide at a constant temperature. It was constructed by placing a $\frac{3}{4}$ inch brass tube concentrically in a $1\frac{1}{2}$ inch (outside diameter) brass tube, fastening this with two brass end pieces, and silver-soldering the ends. The outside tube was tapped near the center with a $\frac{1}{2}$ inch hole into which was screwed a 1 inch tapered brass cup for holding the 8 inch Pyrex reflux condenser. The outside

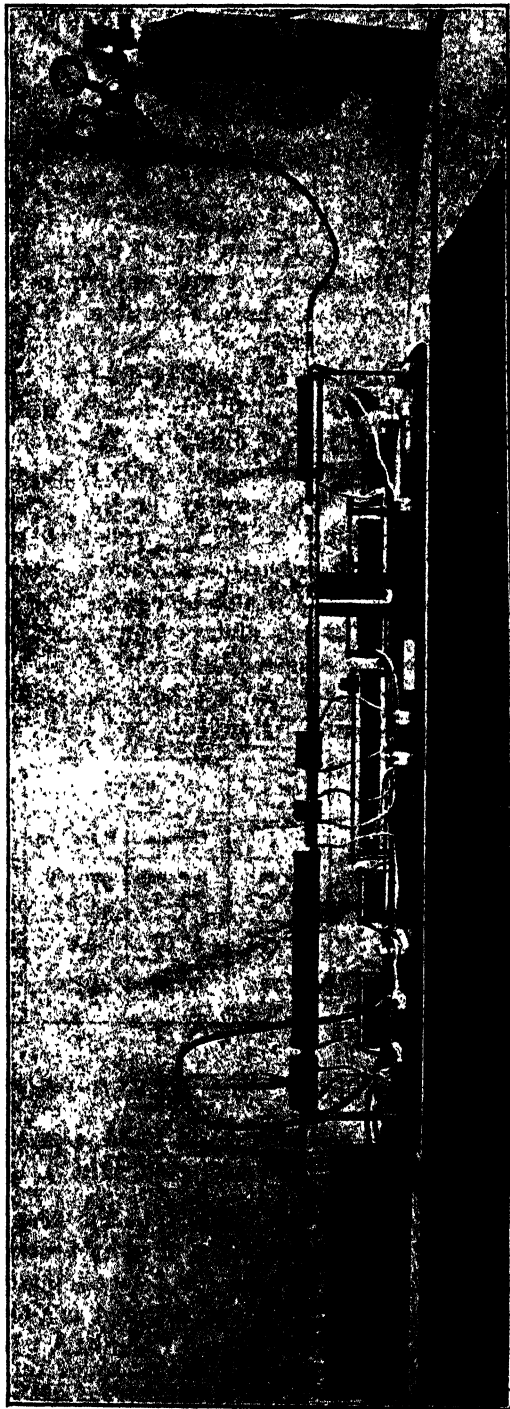


FIG. 2.

tube was covered with mica and wound with 27 feet (26 ohms) of No. 22 asbestos-covered nichrome wire. It was covered with a layer of alundum cement and then wound with asbestos cord. The jacket was filled with sufficient paracymene to cover the inner tube.

Each heating unit is provided with an electric switch, and the temperature is regulated by rheostats, *G*, *H*, *K*, *L*, and *M*; *F* and *N* are two supports (8 inches from the base) for the two parallel bars upon which the heating units rest. *J* is an 8 inch drying tube; one-half is filled with ascarite, and the other half with anhydrous calcium chloride. *I*, which is sealed to *J*, serves as a bubble-counter. It is all mounted on a 12 x 63 inch board.

For carrying out combustions the writers used a Scotch combustion tube 93 cm. long and 1.4 cm. (outside diameter), which was filled as recommended by Pregl¹ ("universal filling"). The preheater tube (36 x 1.4 cm.) was filled with wire-form copper oxide held in place with two oxidized copper-gauze coils. The oxygen for the combustion was obtained directly from a cylinder, which was provided with an efficient reducing valve. During the combustion the various heating units were placed as indicated in Fig. 1. The substance was burned by gradually moving *C* towards *B*, while *D* was always kept over the portion of the tube containing the oxidized copper-gauze coil.

Some typical carbon and hydrogen determinations obtained with this combustion furnace are given as follows:

SUBSTANCE	WEIGHT OF SUB- STANCE	FOUND		CALCULATED	
		Carbon	Hydrogen	Carbon	Hydrogen
	<i>gram</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Sucrose.....	0.0827	42.21	6.51	42.09	6.47
p-Nitraniline.....	0.0894	52.07	4.42	52.16	4.38
2, 3-Dichloroanthraquinone.....	0.0784	60.54	2.28	60.66	2.18

THE YEAST METHOD FOR SILVER PROTEINS.

By H. WALES (Drug Control Laboratory, Food, Drug and Insecticide Administration, Washington, D. C.).

In the course of a recent investigation into the keeping qualities of solutions of the U. S. Pharmacopeia types of silver proteins it was found necessary to study the yeast method for determining the strength of these compounds. This method has been adopted as a tentative method by the Association of Official Agricultural Chemists². Its development

¹ Quantitative Organic Analysis, p. 25. Translated by Ernest Fyleman. J. & A. Churchill, London, 1924.

² This Journal, 1926, 9: 312; 1927, 10: 74, 374.

is due to Pilcher and Sollmann¹, who state that the "content of silver ions * * * is also responsible for all or nearly all of the "antiseptic action on yeast". These authors also comment as follows: "In so far as the antibacterial efficiency of silver compounds is due to silver ions, this would also be faithfully reflected by the yeast method; it would be quite immaterial in principle whether the silver-ion concentration is measured by means of bacteria, or of yeast, or by a chemical or physical method. This, of course, does not mean that the minimum antiseptic concentration is the same for bacteria as for yeast; * * * it means, however, that if silver compound X has a hundred times the efficiency of silver compound Y on yeast, it will also have approximately a hundred times the efficiency on any bacterium".

These statements have recently been refuted by Taylor², who tested a number of preparations against yeast and bacteria and showed that the most efficient compound against bacteria had practically no inhibitory action against the growth of yeast.

In the official method 8 grams of commercial pressed yeast is suspended in 200 cc. of a 10 per cent sucrose solution; 10 cc. of this suspension is placed in each of a series of test tubes with varying quantities of silver protein, and the volume is made up to 20 cc. with distilled water. The tubes are then incubated for 1 hour at 38°C. and examined for gas formation. The concentration of silver protein, which is just sufficient to inhibit the action of yeast, is shown by the tube containing the smallest quantity of silver protein in which there is no gas. The concentration of silver nitrate that will inhibit the activity of the same yeast suspension is determined, and it is assumed that the inhibitory solutions of silver nitrate and silver protein have the same silver-ion concentration.

The yeast used was prepared locally and delivered to the stores in the neighborhood three times a week. It is believed that in no case had the specimens been stored over 3 days. Repeated examinations by W. R. Turner of the Microbiological Laboratory showed the yeast to be a pure culture. Nevertheless in using silver nitrate consistent results could not be obtained for the concentration required to inhibit the growth of yeast suspensions. On the other hand, no difficulty was encountered in obtaining checks for the inhibitory concentrations of the silver proteins. In its action on yeast silver nitrate seems to differ entirely from the silver proteins. The first tubes in the protein series showed large volumes of gas, the next one or two contained no gas but a turbid liquid, and the remaining tubes contained clear liquid and no gas. In the silver nitrate series the volume of gas decreased so gradually from one tube to the next that it was almost impossible to determine in just which tube the growth of yeast had been entirely restrained. When allowed to stand for 24

¹ *J. Lab. Clin. Med.*, 1924, 9: 256.

² *J. Am. Pharm. Assoc.*, 1927, 16: 820.

hours at room temperature all the tubes in the silver nitrate series usually showed gas, the concentration required to kill the yeast being a little higher than that ordinarily used in the series. In the silver protein series the only additional tubes showing gas at the end of the 24 hour period were those that were turbid at the end of 1 hour.

The writer is led to believe from this observation that the inhibitory action of silver protein on the growth of yeast is not due to the concentration of silver ion. The results of potentiometric titrations (to be published) strengthened this belief. Knowledge of the structure of these compounds is too meager at the present time to hazard an opinion in regard to the nature of their action.

NUTRITIVE VALUE OF ALBA BLOOD AS A SOURCE OF PROTEIN¹.

By SIGFRED M. HAUGE (Department of Research Chemistry, Agricultural Experiment Station, Purdue University, Lafayette, Ind.).

Meat by-products such as tankage, meat scraps, and pork cracklings are used as protein concentrates for feeding purposes in the rations of poultry and farm animals. Their value is dependent upon their ability to supplement the proteins of the other constituents of the ration, especially corn. Occasionally attempts are made to use other commercial by-products, high in nitrogen, to increase the protein ($N \times 6.25$) content of these meat by-products. Such a product is "alba blood".

Alba blood first came to the attention of the writer when the State Chemist Department discovered it as a constituent of certain tankages. Investigations disclosed that it is derived from spent printer's rolls. These rolls, which are made from gelatin and glycerol, are subjected to a process whereby all the glycerol possible is removed, the residue being dissolved in water and dried. The dry product is red in color and somewhat resembles dried blood in appearance. Analysis shows that it has approximately the following composition: Moisture 8.1 per cent, protein 82-93 per cent, ash 2.5 per cent, ether extract 0.7 per cent, and fiber 0.4 per cent.

EXPERIMENTAL.

An experiment was planned to determine the biological value of alba blood, both as the sole source of protein in the diet and as a supplement to corn.

The biological tests were conducted with selected albino rats, 50 of which weighed approximately 40-50 grams, and ten of which, used in Lots 2A and 3A, weighed 80-90 grams. Each lot consisted of five rats

¹ Published with the approval of the Director of the Agricultural Experiment Station.

and each rat was placed in an individual cage of a type which prevented access to excreta. The food was given ad libitum in the McCollum-type feeding cups. The composition of the various rations is given in Table 1. Weekly records of the weights of the animals and of the food consumed were kept.

ALBA BLOOD AS THE SOLE SOURCE OF PROTEIN.

With Lots 1, 2, and 3 a study was made of the biological value of alba blood as the sole source of protein in the diet. It was fed at protein levels of 10, 15, and 20 per cent. With Lot 4 this ration was supplemented with gelatin and with Lot 5, with casein, while Lot 6, with 20 per cent casein as the sole source of protein, was used as a check. The results of these tests are shown in Chart I. It is to be noted that alba blood would not support growth or even maintain the animals.

In order to test further the maintenance value of the material, duplicate tests were made of Lots 2 and 3, larger animals, weighing approxi-

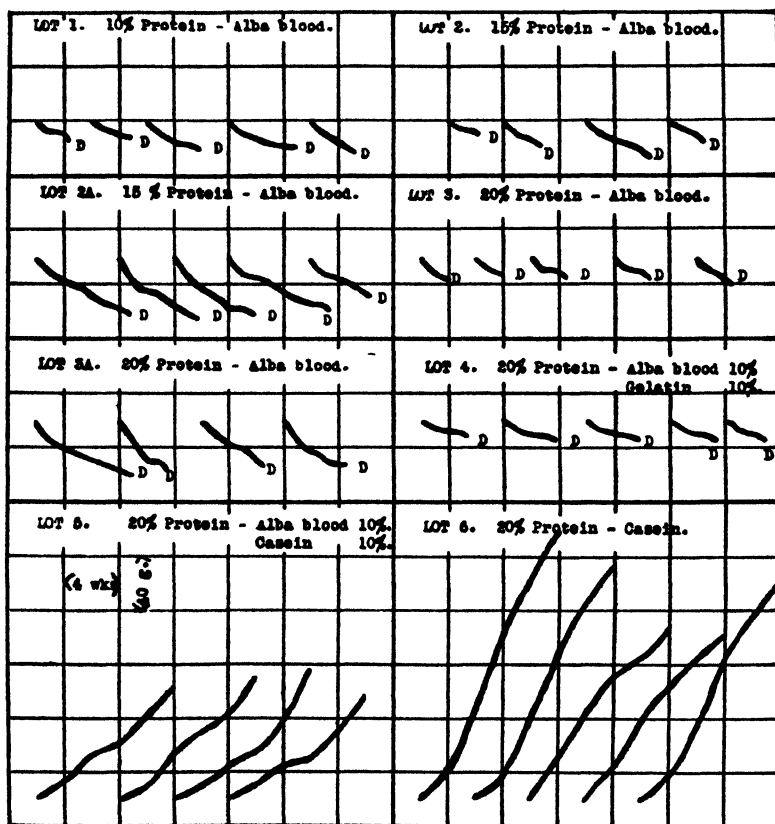


CHART 1. SHOWING THE VALUE OF ALBA BLOOD AS THE SOLE SOURCE OF PROTEIN IN THE DIET.

TABLE 1.
Composition of rations.

Lot No.....	1	2	3	4	5	6	7	8	9	10	11	12
Protein supplement (per cent)....	10	15	20	20	20	20	10	15	20		20	10
Alba blood.....	12.16	18.24	24.32	12.16	12.16		12.16	18.24	24.32		12.16	
Casein.....					10.8	21.7					10.8	10.8
Gelatin.....												
					10.8							
Butter fat.....	8	8	8	8	8	8	8	8	8	8	8	8
C. L. O.*.....	2	2	2	2	2	2	2	2	2	2	2	2
Salt mixture No. 185.....	3	3	3	3	3	3	3	3	3	3	3	3
Yeast.....	3	3	3	3	3	3	3	3	3	3	3	3
Dextrin.....	69.84	63.76	57.68	59.04	59.04	59.3						
Corn.....							69.84	63.76	57.68	82	59.04	71.2
Agar agar.....	2	2	2	2	2	2	2	2	2	2	2	2

* Certified cod liver oil, highly potent in the fat-soluble vitamins, was used in all experiments.

mately 80 to 90 grams, being used. The results of these tests are shown in Lots 2A and 3A, respectively. Although these animals survived longer than the others owing to their greater original weight, they experienced a continuous loss of weight. In fact, in all these tests the greater the quantity of alba blood in the ration, the greater were the deleterious effects. In Lot 4, where 10 per cent alba blood was supplemented with 10 per cent gelatin, no beneficial results were obtained by the addition of gelatin. The supplementing of 10 per cent alba blood with 10 per cent casein resulted in limited growth, as shown in Lot 5. Lot 6, containing 20 per cent casein, gave good growth.

ALBA BLOOD AS A SUPPLEMENT TO CORN.

Although this product proved to be inadequate as the sole source of protein, it was thought possible that it might possess supplementing value to corn. The results of this study are shown in Chart 2. With

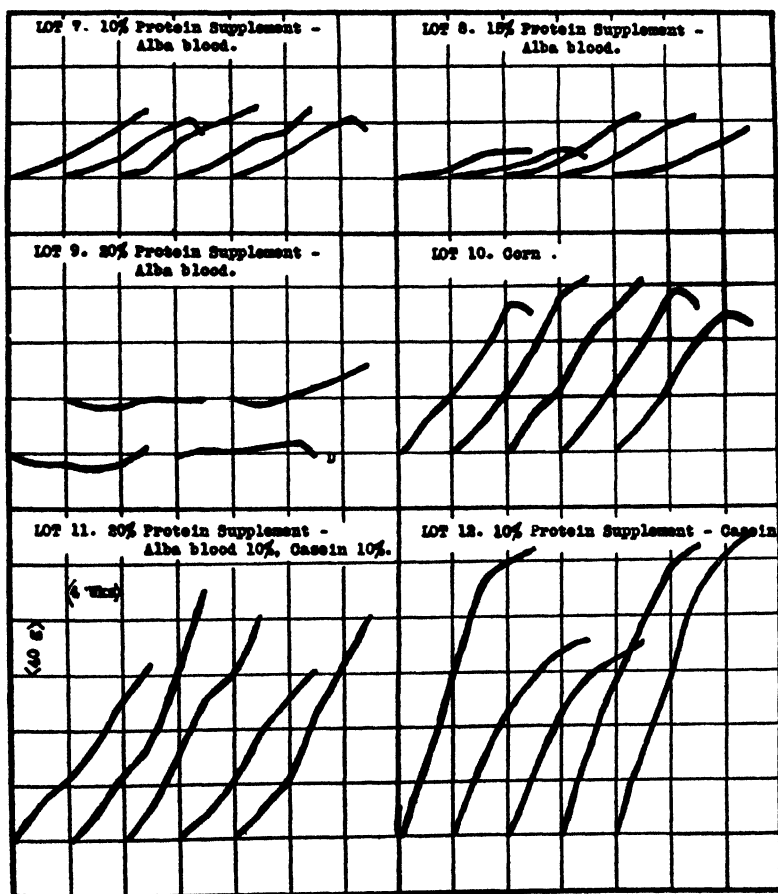


CHART 2. SHOWING THE VALUE OF ALBA BLOOD AS A SUPPLEMENT TO CORN.

Lots 7, 8, and 9 alba blood was used as the sole protein supplement to corn. It will be noted that as the quantity was increased in the ration, the rate of growth was retarded, until in Lot 9, where the protein supplement was fed at a level of 20 per cent protein, growth was inhibited. In contrast to the results recorded for these lots are those of Lots 10, 11, and 12. It was found that corn alone, Lot 10, gave better growth than Lots 7, 8, and 9. When the corn was supplemented by 10 per cent alba blood protein and 10 per cent casein, as in Lot 11, good growth was obtained, but it was not so good as in Lot 12 where the corn was supplemented only with 10 per cent casein.

DISCUSSION.

These experiments show that alba blood is inadequate as the sole source of protein in the diet and also as a supplement to corn.

The nature of the deficiency of this product is indicated by the tests in which gelatin and casein were added to the alba blood. Inasmuch as gelatin did not show any supplementing action, it may be concluded that the deficiencies of alba blood are of the same order as those of pure gelatin. The addition of casein to alba blood resulted in the growth that might be expected from the casein alone, provided the concentrate contained nothing toxic.

The source of alba blood would also indicate that it is principally gelatin or at least closely related to it because printer's rolls are composed chiefly of gelatin and glycerol. Other substances may be used in addition, but without doubt this by-product is composed principally of gelatin.

Since it is well known that the chief deficiencies of the proteins of corn are lysine and tryptophane, the failure of alba blood to supplement corn indicates that it must be deficient in at least one of these amino acids. Gelatin is deficient in tyrosine, cystine, and tryptophane, but according to McCollum and Simmonds¹, to McCollum, Simmonds and Pitz², and to Dakin³, it is rich in lysine and therefore does not supplement corn² because both are deficient in tryptophane. The critical deficiency of alba blood as a supplement to corn probably is tryptophane.

In comparing Lots 7, 8, and 9 with Lot 10, and also Lot 11 with Lot 12, it is seen that the alba blood has a repressing effect on growth. In Lots 1, 2, and 3, the result of increasing the quantity of alba blood was detrimental. It might appear, therefore, that this product contains some toxic factor which interferes with growth and the well being of the animal. However, this conclusion does not seem probable, because in Lots 5 and 11 where casein was used in the presence of alba blood growth

¹ The Newer Knowledge of Nutrition, 3rd ed., 1925, pp. 75, 78.

² *J. Biol. Chem.*, 1917, 28: 486.

³ *Ibid.*, 1920, 44: 499.

was obtained. It would appear, therefore, that the results are due to the deficiencies of the proteins.

The repression of growth by the addition to a diet of a non-supplementing protein is not new. Prange, Hauge, and Carrick¹ found that when gelatin was added to a certain chick ration growth was repressed. From a series of curves, presented by McCollum, Simmonds, and Pitz² in their studies on the deficiencies of corn, it is found that growth was somewhat repressed by the addition of 3.5 per cent of gelatin and still more by 10 per cent. Likewise, Hogan³ found that the addition of zein to a ration of corn and minerals was detrimental. He says: "Again the data are not conclusive but it does seem highly probable that the addition of zein not only had no value, but was really detrimental. * * * It seems highly probable that the addition of zein had actually lowered the value of corn".

To ascribe an explanation for this repressing action of a non-supplementing protein is not feasible at this time. It may be due to the diluting of the ration with a non-essential substance or to the producing of unfavorable metabolic effects with the ration.

Since protein concentrates are purchased mainly for the purpose of supplementing the protein deficiency of corn, the results given in this paper indicate that manufacturers of protein concentrates are hardly justified in adding alba blood or similar materials composed largely of gelatin to meat by-products. Added non-supplementing proteins really act as diluents for the original proteins of the concentrates.

SUMMARY.

1. Alba blood, a commercial by-product recovered from spent printer's rolls, and used to increase the protein ($N \times 6.25$) content of tankages, was tested for its biological value.
2. It was found to be inadequate both as the sole source of protein in the diet and as a supplement to corn.
3. The addition of a non-supplementing protein to the diet appears to have a repressing effect on growth.
4. Results indicate that alba blood should not be used to increase the protein ($N \times 6.25$) content of tankages and meat by-products.

¹ *Poultry Science*, 1927, 6: 306.

² *J. Biol. Chem.*, 1917, 28: 486.

³ *Ibid.*, 1916, 27: 199.

ASSAY OF TRIONAL TABLETS¹.

By L. E. WARREN (U. S. Food, Drug and Insecticide Administration, Washington, D. C.).

At the last meeting of the American Chemical Society at Detroit, the writer presented some observations on the assay of sulfonal in tablets² and proposed two methods of assay which had given good results in a limited number of trials. The procedure in both cases consisted, briefly, in the extraction of the powdered tablet material with a volatile solvent and the evaporation of the solvent without heat. The process took into account the observation of Falck³ that sulfonal is volatile at temperatures above 66°C.

A pharmaceutical manufacturer inquired concerning a method for the assay of trional in tablets. The literature was searched for suitable procedures, but no methods were found. Furthermore, no information was obtained concerning the temperature at which trional begins to volatilize. Since trional is closely related chemically to sulfonal it seemed probable that the substance might be expected to volatilize at temperatures below 100°C. It also seemed probable that the method proposed for the analysis of sulfonal tablets might be applicable for the determination of trional in tablets. It was appreciated, however, that the method was somewhat tedious owing to the necessity of carrying out the evaporation without heat or at temperatures below 60°C. The first consideration in the analysis of trional tablets, therefore, was to determine whether trional solutions in chloroform or other volatile solvent might be evaporated at temperatures in the neighborhood of 100°C. without loss.

A quantity of trional was weighed into a tared beaker, a few cubic centimeters of chloroform was added, and the mixture was agitated until solution had taken place. The mixture was evaporated on the steam bath with occasional rotation of the container until the odor of chloroform had disappeared; the residue was then heated for an hour on the steam bath, cooled, and weighed. The material was again heated on the steam bath, and the residue was weighed at intervals of an hour for 3 hours more. In a second experiment the test was repeated except that the residue obtained by the evaporation of the solvent on the steam bath was dried in the oven at 100°C. The results are given in Table 1.

In order to test further the behavior of trional at temperatures in the neighborhood of 100°C. the following experiment was carried out by two collaborators, N. T. Chamberlin, Western Reserve University, Cleveland, Ohio, and Henry M. Burlage, Board of Pharmacy, Corvallis, Ore.:

¹ Read before the Division of Medicinal Products, American Chemical Society, St. Louis, Mo., April, 1928. Published through courtesy of *Industrial and Engineering Chemistry*.

² *This Journal*, 1927, 10: 523.

³ *Pharm. Zentralhalle*, 1919, 60: 409.

Weigh accurately about 0.3 gram of trional and dissolve the powder in 10 cc. of chloroform. Evaporate the solution to dryness on the water bath and dry the residue at 100°C. in the oven. Note any losses. Repeat the experiment a sufficient number of times to make sure whether any losses occur.

TABLE 1.
Volatility of trional at 100°C.
(Weight expressed in grams.)

METHOD	WEIGHT TAKEN	WEIGHT AFTER HEATING 1 HOUR	WEIGHT AFTER HEATING 2 HOURS	WEIGHT AFTER HEATING 3 HOURS	WEIGHT AFTER HEATING 4 HOURS
Steam bath.....	0.5004	0.4874	0.4659	0.4378	0.4297
Air oven.....	0.5171	0.5085	0.4991	0.4860	0.4652

In these trials Chamberlin reported that 0.3217 gram of trional lost 0.0054 gram, or 1.7 per cent, and that 0.3013 gram of the powder lost 0.0045 gram, or 1.5 per cent. Significant losses were reported by Burlage, but the values were not stated.

These results show that trional volatilizes appreciably at temperatures in the neighborhood of 100°C. Any method of analysis, therefore, which depends on the extraction of the tablet material with a volatile solvent and subsequent evaporation of the solvent cannot make use of temperatures as high as 100°C. in drying the residue after evaporation. This being true, it seemed worth while to ascertain whether other temperatures above room temperature might be safely employed.

Since chloroform boils at about 61°C. the temperature of 60°C. was selected for the first experiment. A quantity of trional was weighed in a tared beaker, a few cubic centimeters of chloroform was added, and the container was placed in a water bath which was maintained at approximately 60°C. for 1 hour. The beaker was then wiped dry, cooled in a desiccator, and weighed. The heating was repeated for an hour and afterward for still another hour. Only a very slight loss of trional occurred. The experiment was repeated with similar results. The same experiments were then conducted at a temperature of approximately 50°C. and also at 70°C. At a temperature of 70°C. a noticeable loss occurred, while at 50°C. the losses were small. The findings for the three temperatures below 100°C. are given in Table 2.

The results of these tests demonstrate that a temperature above 50°C. cannot be employed without loss in drying trional residues after the solvent has been removed by evaporation. However, it was found that dry trional residue did not lose weight when kept over sulfuric acid in a partial vacuum.

Since temperatures of about 50°C. are troublesome to control in a practical way, it was decided in the trial analyses of trional tablets to conduct the evaporations at ordinary temperatures. A mixture consist-

TABLE 2.
Behavior of trional at 50°, 60°, and 70°C.

TEMPERATURE	WEIGHT TAKEN	WEIGHT AFTER HEATING 1 HOUR	WEIGHT AFTER HEATING 2 HOURS	WEIGHT AFTER HEATING 3 HOURS	LOSS AFTER HEATING 3 HOURS
°C.	gram	gram	gram	gram	per cent
50	0.3042	0.3041	0.3037	0.3022	0.66
	0.4911	0.4890	0.4889	0.4888	0.46
60	0.3633	0.3613	0.3589	0.3582	1.4
	0.5658	0.5669	0.5640	0.5636	0.4
70	0.5012	0.4914	0.4834	0.4819	3.8
	0.5636	0.5594	0.5529	0.5492	2.5
	0.3022	0.3009	0.2961	0.2909	3.5

ing of 50 per cent of trional and 50 per cent of starch was first prepared. This was subjected to analysis by each of the following methods:

Method I.

Weigh a sufficient quantity of the powder to represent about 5 grains of trional. Macerate the powder in a small beaker with 10 cc. of chloroform and decant the solvent through a small filter. Repeat the extraction with chloroform until the powder is exhausted of trional. Wash the filter with a few cubic centimeters of fresh chloroform and evaporate the united solvent in a tared beaker at ordinary temperature by the aid of a gentle current of air, taking care near the end of the evaporation to rotate the container in an inclined position. Dry the residue to constant weight in a desiccator over sulfuric acid.

Method II.

Weigh a sufficient quantity of the powder to represent about 5 grains of trional, place it in a fat-free thimble and extract it with chloroform in a Bailey or a Soxhlet extractor until completely exhausted of trional. Evaporate the solvent in a tared beaker at ordinary temperature by the aid of a gentle current of air, taking care near the end of the evaporation to rotate the container in an inclined position. Dry the residue to constant weight in a desiccator over sulfuric acid.

(In both of these methods it is assumed that chloroform-soluble substances other than trional are absent.)

Two commercial brands of trional tablets were received. Portions of these were pulverized in a mortar, and the powder was subjected to analysis by each of the methods described. The results obtained are given in Table 3. The results obtained from the laboratory specimen are also included in this table.

A mixture of trional and starch containing 50 per cent of each was sent to each of several collaborators with the request that the material be assayed for trional by methods essentially the same as have already been described in this paper. Portions of the tablet material previously mentioned were also sent for analysis. The results reported by the collaborators are summarized in Table 4.

TABLE 3.

Results of analyses of trional mixtures obtained by writer.

METHOD	LABORATORY SPECIMEN		SAMPLE A		SAMPLE B	
	Material taken	Theory	Material taken	Claimed	Material taken	Claimed
I	<i>per cent</i> 51.7	<i>per cent</i> 103.4	<i>per cent</i> 73.9	<i>per cent</i> 96.2	<i>per cent</i> 86.9	<i>per cent</i> 97.7
	51.2	102.4	73.2	95.1	86.6	97.3
	51.0	102.0	74.3	96.6		
II	50.7	101.4	74.6	97.0	87.4	98.3
	51.6	103.2	74.6	97.0	87.3	98.1
	51.0	102.0				

TABLE 4.

Results of analyses of trional mixtures obtained by collaborators.

COLLABORATOR	METHOD	LABORATORY SPECIMEN		TRADE SPECIMEN A		TRADE SPECIMEN B	
		Taken	Theory	Taken	Claimed	Taken	Claimed
N. T. C.	I	<i>per cent</i> 50.5	<i>per cent</i> 101.0	<i>per cent</i> 75.0	<i>per cent</i> 96.0	<i>per cent</i> 85.9	<i>per cent</i> 95.7
		50.3	100.6	75.1	96.1	86.5	96.3
		50.3	100.6	75.3	96.4	86.4	96.2
		50.1	100.1	75.6	96.8	86.1	95.9
N. T. C.	II	50.5	101.0				
		50.3	100.6				
K. S.*	I			74.9	97.7	87.5	98.1
				75.1	97.9	88.0	98.9
K. S.	II			76.3	99.2	89.1	100.0
				75.0	97.5	88.3	99.2
H. M. B.	I	49.9	100.2	73.2	95.1	83.8	94.2
H. M. B.	II	57.5	60.0	72.7	94.4	81.6	91.6

* K. O. Sato, H. K. Mulford Co.

CONCLUSIONS.

Two methods for the assay of trional tablets were tried. Each depended on the extraction of the pulverized tablet material with chloroform and evaporation of the solvent without heat. The results obtained by both methods on known mixtures of trional and starch were a little high, averaging about 101.5 per cent of the truth. Extended trials were not carried out, but it is believed that either of the methods may be used in the assay of trional tablets on the market with results that are approximately correct.

The writer wishes to acknowledge his appreciation of the aid received in this brief study from the several pharmaceutical manufacturers and from others who either contributed material or did collaborative work.

APPLICATION TO MEAT AND MEAT FOOD PRODUCTS OF A
RAPID-BOILING SHORT-DIGESTION METHOD FOR
THE DETERMINATION OF PROTEIN.

By H. R. McMILLIN (Meat-inspection Laboratory, Bureau of Animal Industry, Washington, D. C.).

The meat-inspection laboratories of the Bureau of Animal Industry determine the protein in large numbers of samples of meats and meat food products. Under the official methods digestion can hardly be accomplished in less time than $1\frac{1}{2}$ hours, and it often requires 4 hours or more. Since Shedd¹ has recently reported that without loss of accuracy complete digestion may be accomplished by rapid boiling within 20 minutes, the possibility of effecting a material saving in time is evident. Accordingly, the method was tested in order to ascertain its applicability to meats.

EXPERIMENTAL PROCEDURE.

Owing to the necessity—in order to avoid loss of moisture—of mixing samples of meat and meat products rapidly, perfect homogeneity cannot be attained. To compensate for the lack of homogeneity, it was thought advisable to increase the size of the sample. After a number of preliminary experiments to test the principle of Shedd's method the procedure described in this paper was adopted. Four samples of meat products from another experiment, the nitrogen content of which had previously been thoroughly checked by comparative analyses made by 12 skilled analysts, were selected for the first trial of the method.

Sample A—Fresh Sausage.—Approximately 3 grams was placed in an 800 cc. Kjeldahl flask; 0.7 gram of mercuric oxide, 15 grams of powdered potassium sulfate, and 35 cc. of sulfuric acid were added. The flask was placed on a Gilmer electric heater, which had previously been allowed to come to full heat, and digestion was continued for 30 minutes. The acid mixture was practically colorless in 20 minutes, but it was thought desirable to continue the digestion for 10 minutes. After cooling, approximately 500 cc. of water, approximately 2 grams of powdered talcum (to prevent bumping), 85 cc. of a solution containing 25 cc. of a 4 per cent solution of sodium sulfide ($\text{Na}_2\text{S} + 9\text{H}_2\text{O}$), and 60 cc. of a solution of sodium hydroxide (sp. gr. approximately 1.45) were added, the solution being poured down the side of the flask so that it did not mix at once with the acid solution. The flask was then connected with the condenser by means of a Kjeldahl connecting bulb, the tip of which extended below the surface of the standard 0.5 *N* hydrochloric acid in the receiver. The contents were then mixed by shaking and distilled until approximately 200 cc. of distillate had passed over into the receiver.

¹ *This Journal*, 1927, 10: 507.

The excess standard acid was then titrated back with standard 0.1 *N* sodium hydroxide solution, a 2 per cent solution of paranitrophenol in 25 per cent alcohol being used as an indicator.

The results of the determination of the protein in Sample A—Fresh Sausage, as well as in Samples B, C, and D by this method, compared with those by the Gunning method¹, together with the water content of the sample, are given in Table 1.

Sample B—Frankfurt-Style Sausage.—Approximately 2.5 grams was digested and distilled exactly as in the determination of protein in fresh sausage, except that 30 cc. of sulfuric acid was used in the digestion instead of 35 cc. The acid mixture was practically colorless in 15 minutes.

TABLE 1.

Sample A—Fresh sausage.

SAMPLE	GUNNING METHOD		SHORT-DIGESTION METHOD	
	Water	Protein (N × 6.25)	Water	Protein (N × 6.25)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1.....	45.60	11.98	46.05	11.99
2.....	45.73	11.98	46.24	11.85
Average.....	45.66	11.98	46.15	11.92
Average, dry basis.....	22.04	22.13

Sample B—Frankfurt-style sausage.

1.....	64.65	14.80	64.58	14.91
2.....	64.65	14.79	64.65	14.71
Average.....	64.65	14.80	64.62	14.81
Average, dry basis.....	41.86	41.85

Sample C—Canned corned beef.

1.....	56.12	31.66	55.54	32.07
2.....	56.10	31.60	55.48	32.12
Average.....	56.11	31.63	55.51	32.10
Average, dry basis.....	72.04	72.14

Sample D—Dried horse meat.

1.....	5.19	66.20	5.14	66.77
2.....	5.17	66.43	5.08	66.59
Average.....	5.18	66.31	5.11	66.68
Average, dry basis.....	69.93	70.26

¹ *Methods of Analysis*, A. O. A. C., 1925, 8.

Sample C—Canned Corned Beef.—Approximately 2 grams was treated exactly as in the determination of protein in the Frankfurt-style sausage.

Sample D—Dried Horse Meal.—For this sample, which represents dried meats in general and was rich in protein, 1 gram was treated exactly as in the determination of protein in the Frankfurt-style sausage.

The slight variations in the water content shown in Table 1 are explained by the fact that the two sets of analyses were made on different days.

As the method seemed reliable and accurate, additional work was done on routine samples of various sausages, the determinations being made by the regular Gunning method and the short method herein described. Results are given in Table 2.

TABLE 2.
Various kinds of sausage.

KIND OF SAUSAGE	PROTEIN (N \times 6.25)	
	Gunning method	Short-digestion method
	<i>per cent</i>	<i>per cent</i>
Pork.....	10.8	10.73
Pork.....	8.5	8.57
Pork.....	12.77	12.77
Pork.....	8.63	8.8
Bologna-style.....	12.1	12.22
Bologna-style.....	13.6	13.62
Frankfurt-style.....	14.6	14.54
Frankfurt-style.....	13.65	13.74
Frankfurt-style.....	14.46	14.48
Frankfurt-style.....	14.48	14.51
Fresh.....	13.9	13.8
Wiener-style.....	15.0	14.8
Minced.....	14.68	14.79

SUMMARY.

It appears evident from the results of this trial that the rapid-boiling short-digestion method for the determination of protein is applicable to meats and meat food products and that it results in saving from 1 to 3½ hours in the analysis of these products as compared with the official method.

METHODS FOR THE DETERMINATION OF MILK SOLIDS IN MIXED FEEDS.

By A. B. DAVIS (The Hilton-Davis Co., Research Laboratories, Cincinnati, Ohio, in collaboration with The Collis Products Company, Clinton, Iowa.).

One of the present chief sources of feed concentrate in poultry rations is dried buttermilk. Mixed feeds are now on the market containing up

to 20 per cent of this product or other milk solids, and their detection and the estimation of the percentage present is a perplexing problem and one of great importance.

In this paper the various available old as well as the new and original methods have been summarized. Since the milk-sugar and casein content of milk solids varies greatly in products from different sources and both these principal ingredients closely resemble similar materials occurring in grain and other feed ingredients, no highly accurate method would appear to be possible, but by careful work the writer believes that the methods outlined will differentiate milk solids from the sugar and protein of grain of all kinds, fish scrap, meat scrap and all other poultry feed ingredients. The importance of this problem is indicated by the intense interest shown by many agricultural experimental stations throughout the country. While no experiments have been made, it is believed that several of these methods are applicable to the determination of milk solids in many human food products.

A search of the literature pertaining to the properties of buttermilk solids and the separation of the constituents therein from other substances reveals a number of facts upon which may be based methods for the determination of milk solids in mixed feeds of all kinds which are commercially used in compounding such feeds, particularly for poultry¹. These methods may be outlined as follows:

Method No. 1.

Extract the mixed feed first with water, then with acidified alcohol, and finally with clear absolute alcohol. (These extractions remove practically all the vegetable and animal protein other than casein.) Then extract with a borax solution which, after filtering, is acidified to a pH value of 4.7-4.6, using methyl red as an indicator. (This acidity is the optimum for the precipitation of casein, which is then filtered off and determined by its content of nitrogen by the Kjeldahl process.)

Method No. 2.

(Qualitative—to determine the presence of milk solids in mixed feeds.)

This method is based upon the fact that maltose or lactose with ammonium hydroxide produces a characteristic red color. Since no maltose may be expected in any commercial feed ingredient, it is evident that water should extract the milk sugar, which should give a red color with ammonia. Proceed as follows:

Extract 10 grams of the sample with 100 cc. of water and filter. Place 10 cc. of the filtrate in a large test tube along with 10 cc. of 20 per cent ammonium hydroxide solution and immerse the tube in water at about 95°C. If lactose is present, indicating milk solids in the feed, within 20 minutes a pink to red color develops in the tube. If the

¹ This paper was received prior to the publication of a paper by Coe in *This Journal*, 1928, 11: 251.

² Since this work was completed the attention of the writer has been called to Waterman's method, *This Journal*, 1927, 10: 256.

grains themselves should give a pink coloration to the water extract, clarify the extract at 80°C. for a few minutes with 0.5 gram of Darco, or other decolorizing carbon, filter, and proceed as directed previously.

This method has indicated the presence of milk sugar in all mixed feeds which the writer has tried that contained as low as 2 per cent of buttermilk solids.

Method No. 3.

Practically all carbohydrates that may be expected to occur in constituents of mixed feeds give an insoluble osazone, while the osazone of lactose is soluble in boiling water. Several feeds in which the milk solids were determined by this method gave results which are high, but it is believed that the method can be developed according to the following outline:

Extract 20 grams of the feed with 100 cc. of cold water and determine the total sugar in an aliquot of this extract by means of Fehlings' solution. To 25 cc. of the extract add 20 grams of phenylhydrazine and 10 grams of glacial acetic acid. Then add 75 cc. of water, heat the mixture on a steam bath for 2 hours, and filter. The residue represents twice the weight of C₆ sugars contained. The sugars calculated from this weight, subtracted from the total weight determined by Fehlings' solution, represent the quantity of lactose present from which the milk solids are determined by multiplying by 2.5 (assuming 40 per cent of lactose in the milk solids).

The probable reason for this method running high is that the feeds give up other compounds, such as gums and dextrans, which reduce Fehlings' solution and give an abnormally high determination of the total sugars by the method above mentioned.

Method No. 4.

Qualitative.

Method No. 4, a modification of the principles outlined in a paper by S. Levites¹, makes possible the detection of milk solids in small quantities in every commercial feed mixture. A composite sample of approximately 20 feeds was mixed in equal proportions, and 10 grams of this mixture was tested by the following process. Varying quantities of milk solids were then added and mixed, and the test was repeated. Even with low percentages of milk solids added (1-2½ per cent) a much greater volume of precipitate was obtained from the composite sample than was obtained from a blank test on the same feed without the milk solids. The following procedure was followed:

Extract 10 grams of the feed with exactly 100 cc. of a mixture of 79 grams of pyridine and 36 grams of water (commercial pyridine of a boiling point 110°-120°C. is sufficiently good), let settle, and pipet off 25 cc. of the extract. Now add 50 cc. of pyridine. (If milk solids are present, a voluminous precipitate is obtained in a few minutes.) Pipet an additional 25 cc. of extract and acidify with hydrochloric acid to a pH value of 4.7-4.6

¹ *Z. Chem. Ind. Kolloide*, 1911, 8: 4; *C. A.*, 1911, 5: 2445.

(methyl red as an indicator and acidified to the point where a magenta color just develops). In a few minutes, if milk solids are present, a voluminous precipitate is obtained, the volume of which is directly proportional to the amount of milk solids.

On all blank tests run small precipitates were obtained, but on a composite sample of 20 feeds the precipitate was approximately equivalent to one-fifth of that obtained when the same sample contained $2\frac{1}{2}$ per cent of milk solids. This method is capable of quantitative estimation down to low percentages.

Method No. 5.

This method is based on the principles given in the abstract of a paper by Baker and Hulton¹, which are corroborated by other references in the literature. The method is as follows:

Extract 10 grams of mixed feeds with 100 cc. of water, filter, and ferment the filtrate for 20 hours at about 27°C. by the addition of 0.2 gram of Fleischmann's yeast and a crystal of sodium phosphate. Filter or centrifuge, and determine lactose by use of Fehlings' solution. Calculate the quantity from the weight of copper oxide, as given in the tables of the A. O. A. C.²

All grain sugars are converted into alcohols, while the milk sugar remains practically unchanged. Blanks run on commercial buttermilk solids by this method will give a lactose factor which is used in the calculation. This factor may vary from 29 to 38 per cent, according to the condition and the purity of the yeast.

Five samples of feed run in the writer's laboratory by this method with unknown samples of feeds were found after analysis to check against the known content of buttermilk solids as follows:

SAMPLE NO.	KNOWN CONTENT	DETERMINED
	<i>per cent</i>	<i>per cent</i>
1	20	17.88
2	5	6.12
3	22	20.4
4	35	38.4
5	42	42.8

These determinations indicate that this method is sufficient for all practical purposes.

Method No. 6.

Inasmuch as such products as meat scrap, fish scrap, and other bodies of this character are found to carry practically no readily extracted fermentable material, it is evident that the grains are the only source of fermentable bodies which would contaminate the water extracts of the feeds. Extracts of several mixed grains gave a constant factor of 0.00134 gram of copper oxide per cubic centimeter of extract by Fehlings' solu-

¹ *Analyst*, 1910, 35: 512; *C. A.*, 1911, 5: 929.

² *Methods of Analysis*, A. O. A. C., 1925, 434.

tion when 10 grams of feed was extracted with 100 cc. of water. The following method may be used:

Extract 10 grams of the mixed feed with 100 cc. of water. Pipet out 25 cc. of the extract and determine the total sugar and reducing compounds direct by means of Fehlings' solution. From the weight of copper oxide obtained, deduct the quantity (cc.) of extract used times 0.00134. The resulting weight of copper oxide then represents the total reducing compounds less reducing compounds other than lactose. This weight, referred to the A. O. A. C. tables, gives total weight of lactose in the volume of extract used. If 25 cc. is used, this weight times 4 will equal the total amount of lactose in the feed, which product multiplied by $2\frac{1}{2}$ will equal the percentage of milk solids.

By this method three samples of unknown feeds were run. The determinations were checked against the known content of buttermilk solids in the feed with the following results:

SAMPLE NO.	KNOWN CONTENT	DETERMINED
	<i>per cent</i>	<i>per cent</i>
1	12	12.24
2	28	29.8
3	48	49.4

Method No. 7.

In considering the various factors that might influence the accuracy of Method No. 6, all the common grains were extracted to determine the amount of reducing substance washed out; the factors obtained showed that while the figure 0.00134 given in Method No. 6 was approximately accurate for most ordinary grain mixtures, such individual grains as alfalfa, red dog, flax meal, and standard wheat middlings gave higher factors.

It is well known that the small amounts of reducing substances that are extracted from grain consist largely of oxycarbohydrates similar to the vegetable gums, and that these are precipitated almost entirely by neutral lead acetate. It is obvious, therefore, that by precipitating these reducing substances with lead acetate, which does not precipitate any lactose, the necessity for the use of a deduction factor is eliminated. The following method was then formulated:

Place 8 grams of sample in a 200 cc. graduated flask with 1-3 grams of calcium carbonate to neutralize any acid. Add 100 cc. of 50 per cent alcohol and boil for $\frac{1}{2}$ hour on the water bath. Allow to stand for several hours. Make up to volume with 95 per cent alcohol and filter. Pipet 100 cc., evaporate to 15-20 cc. on the steam bath, and transfer to a 100 cc. graduated flask. - Add 5 cc. of saturated neutral lead acetate to produce a flocculent precipitate, shake well, and allow to stand 15 minutes. Make up to volume with water and filter through a dry filter. Add $\frac{1}{2}$ gram of sodium carbonate to precipitate excess lead and filter through a dry filter. At this point test the filtrate by adding a little more sodium carbonate to see that all the lead has been precipitated. Now run Fehlings' test for quantity of sugar, using 25 cc. of the filtrate; 25 cc. of filtrate is

equivalent to 1 gram of sample. Weight of Cu_2O times 0.8882 gives weight of copper, from which the corresponding weight of lactose is found in the Soxhlet-Wein table¹.

$$\frac{\text{Lactose}}{36} \times 100 = \text{percentage of milk solids.}$$

The figure 36 is the buttermilk factor run on buttermilk solids according to this method.

As a check on Method No. 7, the following tests were made:

KIND OF FEED	Cu_2O gram	LACTOSE per cent	B. M. S. per cent
1. Straight buttermilk solids.....	0.2755	36
2. Mixed grain containing fish and meat scrap with 12½ per cent buttermilk solids.....	0.0700	..	12 7
3. Mixed grain containing fish and meat scrap with 20 per cent buttermilk solids	0.1140	..	20.1

A series of samples consisting of several grains, milk solids, and various protein-bearing material gave the following results, which were checked against the known content as later disclosed:

ANALYSIS per cent	KNOWN CONTENT per cent
11.11	12.5
27.56	31.3
45.02	46.8
61.64	62.0
92.50	93.8

One sample gave 20.2 per cent buttermilk solids; this sample contained 20 per cent buttermilk solids.

APPLICATION OF METHOD No. 7 TO THE DETERMINATION OF DRIED SKIM MILK IN FEEDS.

When dried skim milk is used in place of dried buttermilk in mixed feeds, Method No. 7 may be used to determine the quantity present. The only variation is in the computation of the results, a factor of 50 being substituted for 36 in the formula—

$$\frac{\text{lactose}}{\text{factor}} \times 100 = \text{percentage of milk solids.}$$

The factors 36 and 50 used in the determination of dried buttermilk and dried skim milk solids represent the percentage of lactose in normal samples of buttermilk or skim milk, respectively. Throughout the year the milk may vary in lactose as much as 10 per cent, due to the changes in feed rations from green pasture to dried winter feeds. As a rule, however, it has been found that the average dried milk will seldom vary

¹ *Methods of Analysis*, A. O. A. C., 1925, 452.

more than enough to throw Method No. 7 off more than a small percentage. When decomposition has taken place or the sugar has been broken down by enzyme action, after a long period of storage, these factors must be reduced in accordance with the condition of the feed.

A mash sample with a content of dried skim milk of 20.0 per cent was found by Method No. 7 to contain exactly 20.0 per cent of dried skim milk on an average of two tests showing 19.8 and 20.2 per cent. Many tests on samples containing 2-20 per cent milk solids gave results of sufficient accuracy for all practical purposes on a great variety of feeds.

Method No. 8.

The following process devised by the writer to indicate the presence of buttermilk solids in feeds is based upon the formation of a blue coloration, the intensity of which is influenced by the amount of buttermilk solids (casein) present.

Treat a 5 gram sample of feed with 30 cc. of water and 2 grams of powdered lime (CaO). After shaking well add 5 cc. of 20 per cent copper sulfate solution and again agitate the mixture. Pour the material into test tubes and allow to stand. Immediate observation and comparison is made against mixtures treated similarly, which have a known buttermilk content. After 2 days, observations and comparisons are again made.

Six samples were prepared containing varying percentages of buttermilk solids, as follows: (1) No buttermilk solids; (2) $2\frac{1}{2}$ per cent; (3) 5 per cent; (4) $7\frac{1}{2}$ per cent; (5) 10 per cent; and (6) 15 per cent.

Upon immediate examination Sample No. 1 was found to be dark blue, while No. 6 was a very light blue. The intermediate samples gave degrees of color which were proportionate to the buttermilk solids present, the color decreasing in intensity as the percentage of buttermilk solids increased.

After two days the material in the test tubes had separated into a liquid and a solid layer. The solid layer in Sample No. 1 was still dark blue in color; Samples 2, 3, 4, 5, and 6 were increasingly lighter and more yellow; while No. 6 showed no sign of blue at all.

By this method unknown samples run at the same time with samples of feeds having known buttermilk solids content can be compared with them, and an estimate of the percentage of buttermilk solids can be made.

Method No. 9.

A report by Howard E. Gensler on the microscopic detection of milk solids in feeds has been published recently¹. The method is rapid and practicable for qualitative work, but it gives little idea of the quantity of milk solids present. It should be valuable where low percentages of milk solids are to be determined or where the quantity is so small that

¹ *This Journal*, 1928, 11: 155.

other methods would leave in doubt the question of the presence of milk solids.

GENERAL CONCLUSIONS.

It is evident from the results given in this paper that either the fermentation method, No. 5, or Method No. 7, by means of Fehlings' solution, is sufficiently accurate for the determination of buttermilk solids in all mixed feeds where the approximate percentage of lactose in the milk solids is known, and that Method No. 1 is an accurate method for the detection of the presence of buttermilk solids in mixed feeds down to small percentages, on the basis of the separation of the casein from other proteins.

Method No. 2 may be accepted for determining the presence of milk solids, but if the quantity present is indefinite or the percentage is low, the results should be checked by some of the other methods.

Methods 5, 6, and 7 are regarded as entirely practicable, and if the percentage of carbohydrates in the milk solids is known or if a sample of the buttermilk solids used can be obtained for accurately determining the buttermilk factor, Method 7 is considered the most accurate.

The experience of the writer and the general consensus of opinion of the collaborators show that Method 9 is a practicable and reliable procedure for detecting dried buttermilk in feeds. Any error which may occur may be attributed to the personal factor of the analyst.

BOOK REVIEWS.

Oils, Fats and Fatty Foods. By E. RICHARDS BOLTON. With a chapter on vitamins by Professor J. C. Drummond. XVI—416 pages. Second edition on **Fatty Foods** by E. R. Bolton and C. Revis. P. Blackiston's Son & Co., Philadelphia, 1928. Price \$8.00.

The plan followed in the original volume, which appeared about 14 years ago, has been adhered to as closely as possible in the present work, but the number of chapters has been increased from 9 to 14. The chapter headings are as follows: 1—General Introduction; 2—Preliminary Examination; 3—General Analytical Methods; 4—Interpretation of Analytical Methods for Oils and Fats and the Analyses of Typical Samples; 5—Industrial Production of Vegetable Oils and Fats; 6—Butter and Margarine; 7—Animal Fats, Fish and Marine Animal Oils; 8—Vegetable Oils and Fats; 9—Hydrogenation of Oils; 10—Rancidity, Preservatives and Colouring Matters; 11—Cocoa, Chocolate and Milk Chocolate; 12—Feeding Stuffs; 13—Milk; and 14—The Nutritive Value of Edible Oils and Fats.

The volume is unique in that the analytical data given for the various fats and oils were obtained for the most part in the author's laboratory by the methods that he describes. The author assumes that the reader has a working knowledge of the chemistry of fats and oils; consequently he describes manufacturing operations briefly and in most cases gives for each test a single method that is based upon his laboratory experience. The idea of a given method for each test is to be strongly commended, provided the best methods are selected. With a few possible exceptions, the author has selected these methods well. The reviewer believes, however, that the André-Cook method for the acetyl value, the Steele and Washburn method for the hexabromide value, and the Kerr-Sorber method for the unsaponifiable matter are superior to the procedures described. Also the Crismer value method is preferable to the Valenta. As the Butyro scale is but little used in this country a table of the refractive indices as determined by the Abbé refractometer would have been a useful addition to this work. In view of the investigations of Power and Tutin, of Stewart, and of the recent study of Allan and Moore, the author has placed too great stress on the value of the phytosteryl acetate test when applied to the detection of animal fats in mixture with those of vegetable origin.

The treatment of some of the animal fats, and of the fish and marine animal oils could have been made more comprehensive. No mention is made of the recent but important pilchard fish oil industry on the American Pacific Coast.

Mr. Bolton is to be congratulated upon the completion of this readable and useful work. The clear direct manner in which he has treated the subject matter is noteworthy. Only one who has made an intimate study of such a large number of the fats and oils, and of their manufactured products could hope to write a book of this outstanding character. Professor Drummond, who contributed Chapter 14, has also succeeded admirably in his presentation, within comparatively few pages, of all the more important facts respecting the so-called vitamins and their functions.

Mr. Bolton has exercised great care in the arrangement of his subject matter as well as in the preparation of the botanical and subject indices. The plates picturing the various fruits and seeds are excellent. He has wisely called attention to the confusion that exists in regard to the botanical classification of various members of the palm family, and also states that in a few other families a revision of these botanical classifications may be necessary. Praise is due both to the proof readers and the publishers for their part in the production of this volume.—G. S. JAMIESON.

The Analytical Detection of the Bleaching of Wheat Flour. Summary in English. By HOLGER JORGENSEN, Copenhagen, 1928.

This paper-covered booklet consists of some 40 pages of Danish text and a 22 page summary in English. Five tables of analyses accompany the Danish and four the English section.

The discussion includes the natural color of flour; the four chief agents commonly used in bleaching: nitrogen peroxide, chlorine, nitrogen-trichloride, and benzoyl-peroxide; the action of these agents upon the carotin or coloring matter of flour; the attitude of officials of various governments toward bleaching; the laws that have already been enacted in this regard; a statement of the objects of the research, viz., to solve the problem of analytically determining whether a flour has been bleached and the nature of the bleaching agent.

No method has as yet been evolved to determine with certainty the nature of each of these bleaching agents. This is especially true with respect to benzoyl peroxide. As only 13 grams of this bleaching agent (containing 16 per cent benzoyl-peroxide) are used to bleach 100 kg. of flour, the amount of benzoic acid present in flour cannot exceed 1 part per 50,000, the detection of which has thus far baffled all efforts.

The nitrogen peroxide is detected by the well-known Griess-Ilosvay method and chlorine by the presence of chlorine in the fat, though in the case of flour bleached with nitrogen trichloride it is a most difficult matter to establish that fact unless the flour has been heavily treated. The quantitative determination of both nitrogen peroxide and chloride is carried on in accordance with methods essentially the same as those approved by the Association of Official Agricultural Chemists.

The methods used to determine moisture, ash, and fat differ widely from those used here. No American chemist would choose to determine moisture in flour by drying 8 grams in a partial vacuum at 80°–85°C. for 16 hours; nor would he burn 20–50 grams of flour to estimate the ash, nor extract 40 grams with gasoline to obtain the fat.

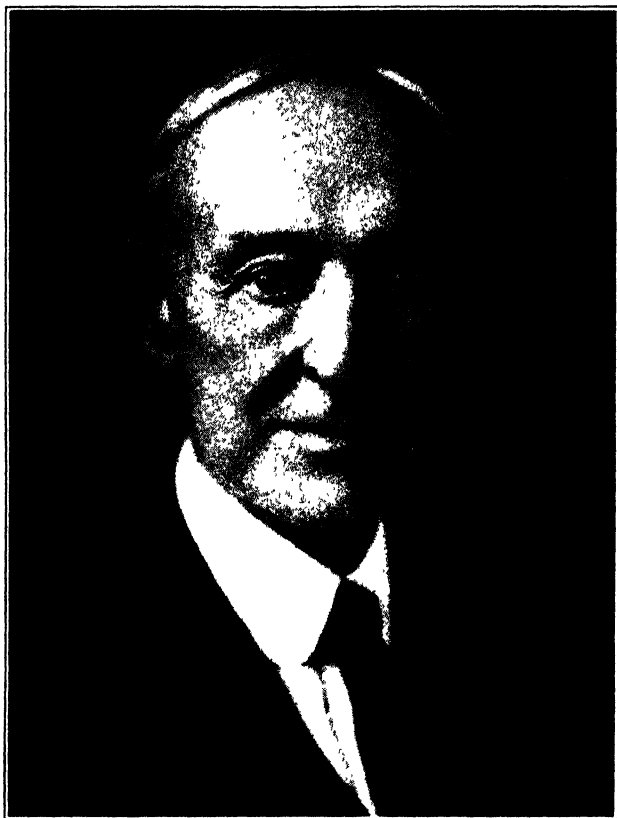
The real contribution consists in the emphasis placed upon the gasoline value and the conclusion that any flour with a gasoline value of 90 or less has been bleached. Winton's method, with slight modifications, has been used. Winton's color value of 1 is taken by the author as 100. On this basis every sample of unbleached flour analyzed (66 of them) and retested at the end of 6 months showed a color value of 95 or more. Every sample having a gasoline value of 90 or less and condemned as bleached, had in fact been treated.

To determine the gasoline value, a buffer solution with a pH of 5.6, made up by mixing two of Sorensen's standard solutions of "prim phosphate" and "sec phosphate" in the proportion of 95 volume parts of the former to 5 volume parts of the latter, is used with the standard potassium chromate.

A flour containing as much as 15 mg. of chlorine or 40×10^{-4} grams of nitrogen peroxide per kilo may be declared to have been bleached.

It is a well-known fact that flour will occasionally absorb more nitrogen peroxide than the standard above given. American flours, unbleached, have likewise been noted to have a lower gasoline value than 90.

Altogether this is an interesting and important contribution to the subject.—J. A. LEClerc.



HENRY CLAY WHITE, 1848-1927.

HENRY CLAY WHITE

The death of Dr. Henry Clay White of Athens, Georgia, on December 1, 1927, marks the passing of one of the founders of the Association of Official Agricultural Chemists, of whom only a very few representatives are now living.

Dr. White was born at Baltimore on December 30, 1848. His chemical training was obtained under Prof. J. W. Mallet at the University of Virginia, from which institution he obtained his bachelor's degree in 1870 and the degree of Ph. D. in 1875. From 1870 to 1872 he taught chemistry at the Maryland Institute, the Peabody Institute of Baltimore, and St. John's College of Annapolis. In 1872 he was appointed Professor of Chemistry at the University of Georgia, and his connection with this institution continued during the remainder of his life.

From the very commencement of his scientific career Dr. White was interested in the application of chemistry to agriculture. In 1874 he published his "Complete Chemistry of the Cotton Plant", a work which remained for many years the most exhaustive treatise upon the subject. In 1880 Dr. White, in addition to his duties at the University, was appointed State Chemist of Georgia, the functions of which office he discharged with great credit during the next ten years. One of the duties of this position was the regulatory control of the purity of the fertilizers which were sold to the planters of Georgia, and it was as a result of this activity that Dr. White became interested in the movement to establish a society of agricultural chemists.

As early as 1880, while I was still a professor at Purdue University and somewhat out of touch with pending developments, the need of such an organization began to be keenly felt. The Hon. J. T. Henderson, Commissioner of Agriculture of the State of Georgia, was the first person to call attention to this need; his action, which was warmly supported by Dr. White, was perhaps the result of a suggestion made either by Dr. White or by H. J. Redding, afterwards Director of the Georgia Agricultural Experiment Station.

Through the courtesy of Hon. William G. Le Duc, Commissioner of Agriculture, this first meeting of American Agricultural Chemists was held in the library of the main building of the Department of Agriculture in Washington.

The proceedings of this early meeting, which was destined to have such far-reaching consequences, are summarized in my "Historical Sketch of the Association of Official Agricultural Chemists"¹, presented at the Sixteenth Annual Convention in San Francisco, July 5, 1899. Dr. White took an active part in the work of this first meeting in which reports were presented for the estimation of nitrogen, potash, and phosphoric acid in commercial fertilizers. He was also an active participant at the second meeting of the association held in Boston the same year and at the third meeting held in Cincinnati on August 18, 1881. I attended the general convention of the American Association at Cincinnati, but on the day of the meeting of the agricultural chemists I was unable to leave my room because of illness and therefore did not participate in its deliberations.

After a dormant period that lasted nearly three years Commissioner Henderson of Georgia issued another call for the agricultural chemists to meet in the senate chamber of the capitol at Atlanta on May 15, 1884.

¹ U. S. Dept. Agr. Div. Chem. Bull. 57, p. 16.

This meeting was the precursor of the present organization of Official Agricultural Chemists. Three committees were appointed on methods of analysis. Dr. White served with S. W. Johnson and W. C. Stubbs on the Committee for Determining Phosphoric Acid. The Committee on Nitrogen consisted of P. E. Chazal, A. T. Neale, and J. A. Myers, and that on Potash of E. H. Jenkins, W. J. Gascoyne, and H. W. Wiley. It is worth noting that the members of the Committees on Phosphoric Acid and Nitrogen have all passed away, while those on the Committee on Potash are still living.

When the Atlanta convention adjourned, the members agreed to meet again the following September with the American Association for the Advancement of Science at Philadelphia. At this meeting, which took place on September 8, 1884, it was the unanimous opinion that the proposed organization of agricultural chemists should be entirely separate from the American Association. Dr. White was appointed chairman of a committee, consisting of P. C. Chazal, E. H. Jenkins, J. A. Myers and H. W. Wiley as the other members, to draw up plans of organization. The report of this committee, which was presented on September 9, forms the basis of the constitution of our society as it exists today, and thus the Association of Official Agricultural Chemists was started upon its long and successful career.

At the second annual meeting of the association, which was held in the library at the main building of the Department of Agriculture in Washington on September 1, 1885, Dr. White, who was vice-president, presided in the absence of the president, Prof. S. W. Johnson.

The last annual convention of the association which Dr. White attended was the Washington meeting of August 9 and 10, 1888. In 1890 he was appointed President of the University of Georgia, and from this time on his chief activities were in the field of education, in which he rendered most distinguished service. He was president of the Association of the American Agricultural Colleges and Experiment Stations in 1897-98 and was a member of the American Chemical Society, of the Chemical Society of London and of the American Association for the Advancement of Science. He was also a corresponding member of the British Association for the Advancement of Science and an honorary member of the Academy of Science of Belgium. Besides his publication "Complete Chemistry of the Cotton Plant", he was the author of two volumes of "Lectures and Addresses", of a treatise on "The Manuring of Cotton", and of numerous bulletins and miscellaneous articles. An account of his collaborative scientific work with the U. S. Department of Agriculture and with the Office of Experiment Stations is contained in an editorial in the Experiment Station Record for April, 1928.

Dr. White resigned the presidency of the University of Georgia in 1907 and his position as chemist of the Georgia Agricultural Experiment Station in 1914, but notwithstanding his advancing years he continued his work as Professor of Chemistry at the University almost until the very end.

Although Dr. White's major activities in later life were diverted from the field of agricultural chemistry, he always continued to maintain an interest in this subject and in the work of the Association of Official Agricultural Chemists, which he was so instrumental in helping to establish. Those of us who were his associates in the early meetings of our association remember him as a most agreeable gentleman, a good comrade, and an industrious worker. His long career has had a most beneficial influence upon the development of scientific agriculture in America.

H. W. WILEY.

SECOND DAY.

TUESDAY—MORNING SESSION.

REPORT ON CHEMICAL REAGENTS.

By G. C. SPENCER (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

Instead of reporting the statistical facts about chemicals that have been rejected by the Bureau of Chemistry and by its successor, the Food, Drug and Insecticide Administration, this past year, it is proposed to describe briefly a method for estimating the amount of sulfate which may be present in such a compound as ammonium or sodium nitrate.

It is well known that barium sulfate is not readily precipitated in the presence of nitrates. It became necessary, therefore, during the study of ammonium nitrate specifications, to devise a method for removing the nitrates without employing the usual evaporation procedures, which are time-consuming and frequently unreliable owing to spattering.

The procedure adopted was as follows:

Transfer 5 grams of the nitrate sample to a 500 cc. Kjeldahl flask (not previously used for sulfuric acid digestions) and add 20 cc. of concentrated hydrochloric acid. Digest on a steam bath or over a low flame. Renew the hydrochloric acid lost by evaporation and continue the heating till a piece of starch-iodide paper is only slightly colored by the fumes. Transfer the mixture from the flask to a 100 cc. beaker with a little water and evaporate to dryness on a steam bath. Take up in a little water and 2 cc. of concentrated hydrochloric acid, filter if necessary, and precipitate the sulfate in the usual manner.

This method has been published¹ under the caption, "Ammonium Nitrate".

It is hoped that this reminder may be of interest and possibly of use to some of the chemists present.

REPORT ON EGGS AND EGG PRODUCTS.

By J. C. PALMER (U. S. Food, Drug and Insecticide Administration, Seattle, Wash.), *Referee.*

Last year work was recommended on the perfection of methods for water-soluble protein-nitrogen precipitable by 40 per cent alcohol, ash, and unsaponifiable matter, and if possible, the collaborative study of these methods together with the present methods for fat, acid hydrolysis, lipoids, and lipoid phosphoric acid. It was anticipated that the first

¹ *Ind. Eng. Chem.*, 1927, 19: 645.

three methods would be developed sufficiently to submit them to collaborative study together with the three last-mentioned methods, but sufficient time was not found to devote to them. Some work was done on the water-soluble protein-nitrogen method by the referee, and Associate Referee Alfend submitted a report on ash. Some work was also accomplished on the solids determination, but no further developments can be reported on methods for the detection of decomposition.

WATER-SOLUBLE PROTEIN NITROGEN.

This determination always has been difficult owing to the filtration of both the water suspension and the precipitated albumen, especially in the case of liquid eggs. The method was carefully studied, but no degree of success was reported on liquid eggs. The filtrations of dried eggs are not objectionable, and it is thought that if more time is spent on the liquid egg filtrations an improvement can be effected.

TOTAL SOLIDS.

The problem in regard to total solids was to determine the loss of material other than water when egg products are dried at temperatures higher than 55°C. It was thought that if the assumption is true that egg proteins and fats are volatilized at 100°C. whole egg, dried at 55°C., and later at 100°C., would show a loss of lipid or nitrogen content. Accordingly, a sample of fresh whole eggs was well mixed and dried in vacuum at 55°C. to about 4 per cent moisture content. The sample was then finely ground and well mixed, and several 2 gram portions were weighed out. Moisture was determined on part of them by the 55°C. vacuum method and on a like number by the 100°C. vacuum method. The samples were then removed from the oven and used for the lipid and nitrogen determinations. Since the samples were of uniform character when they were put in the oven, it was assumed that any loss shown in lipid or protein, on the sample heated to 100°C., could be ascribed to the action of the heat on the volatile principle. The results are shown in the tables.

The results in Table 1 show that very little, if any, loss of nitrogen or lipid occurs in the samples heated to 100°C. The moisture content is over 1 per cent higher by the 100°C. method, which indicates the failure of the 55°C. method to remove all the moisture present.

Table 2 gives comparative results obtained for moisture content on various egg products by various methods. It is not known to what temperature the albumen and yolk were exposed during drying, but it is assumed that this temperature was not more than 60°C. since the manufacturing processes used in commercial drying are designed to prevent coagulation. The distillation method for testing moisture gives results quite comparable to those obtained by the 100°C. vacuum method in

TABLE 1.
Analysis of whole egg.*

	DRIED AT 55°C. VACUUM	DRIED AT 100°C. VACUUM
	<i>per cent</i>	<i>per cent</i>
Nitrogen.....	7.48	7.46
Lipoids.....	44.87	44.80
Moisture.....	3.93	5.10

TABLE 2.
Comparison of moisture methods on egg products.

	55°C. VAC.	100°C. VAC.	DISTILLATION WITH XYLENE, 106°C.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Whole egg*.....	3.93	5.10	5.30
Powdered spray yolk†.....	2.87	3.87	3.75
“ albumen†.....	14.84	16.34	16.36

* Fresh eggs, reduced to solid condition by heating in vacuum at temperature of 55°C.

† Commercial samples, temperature of drying unknown.

the case of most food products. Its application to egg products shows similar results. The moisture content of both the yolk and albumen by the two methods is in close agreement. However, the whole egg shows a greater loss when exposed to the higher temperature of the xylene.

ACKNOWLEDGMENT.

The chemical work on total solids reported, with one exception, was performed by C. E. Goodrich, Food, Drug and Insecticide Administration, Washington, D. C. The work with the distillation method was done by G. L. Bidwell of the same Bureau.

RECOMMENDATIONS¹.

It is recommended—

- (1) That further study, accompanied by collaborative work if possible, be made of the following determinations:
 - a. Water-soluble protein-nitrogen precipitable by 40 per cent alcohol and
 - b. Unsaponifiable matter.
- (2) That collaborative study be made of the following determinations:
 - a. Ash,
 - b. Fat, acid hydrolysis,
 - c. Lipoids, and
 - d. Lipoid phosphoric acid.
- (3) That study of methods for detection of decomposition be made in:
 - a. Acid-soluble phosphoric acid,
 - b. Ammonia nitrogen, and
 - c. Reducing substances as dextrose.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 79.

REPORT ON WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, AND ASH IN EGGS.

By SAMUEL ALFEND (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.), *Associate Referee*.

Several methods were recommended for individual study this year, but owing to conditions in the associate referee's laboratory only the development of a satisfactory method for the determination of ash was undertaken.

The determination of ash in liquid eggs was first studied. A series of determinations was made in flat-bottomed silica dishes $7\frac{1}{2}$ cm. in diameter. Twelve samples of approximately 2 grams each were weighed out. Samples 1 and 2 were evaporated over a small Bunsen flame on an asbestos-centered iron gauze, charred, and heated in a muffle at low red heat until they ceased to lose weight. About 1 gram of 60-mesh alumina (alundum), accurately weighed, was added to each of Samples 3 and 4, which were then ashed in the same manner as Samples 1 and 2. Samples 5 and 6 were treated with 2 cc. of 10 per cent magnesium acetate solution, evaporated over a small flame, charred, and ignited in a muffle at low red heat to constant weight. Blank determinations were run on the magnesium acetate solution. Samples 7 and 8 were treated with 10 cc. of a solution containing glycerol and alcohol in equal proportions. When ignited these samples frothed over and were lost. The experiment was repeated, the mixture first being evaporated on the steam bath and then heated cautiously over a small flame. Again frothing

TABLE 1.

Results of ashing liquid eggs in silica dishes by various methods.

SAMPLE NO.	TOTAL HEATING TIME IN MUFFLE	ASH	COMMENTS
	<i>hours</i>	<i>per cent</i>	
1	10	0.842	Creeping and slight spattering during charring.
2	9	0.835	
3	6	0.848	Slight creeping and spattering during charring.
4	6	0.844	
5	5	0.958	No spattering or creeping.
6	$5\frac{1}{2}$	0.961	
7	Samples lost due to frothing.
8			
9	3	0.848	No spattering; slight creeping.
10	3	0.849	
11	$2\frac{1}{2}$	0.968	No spattering or creeping.
12	$2\frac{1}{2}$	0.968	

resulted, and most of the sample was lost. Samples 9 and 10 were treated in the same manner as Samples 3 and 4, except that 5 grams of alundum was added. Samples 11 and 12 were treated with 3 grams of alundum and 2 cc. of 10 per cent magnesium acetate solution.

The results obtained, with the comments on the determinations, are given in Table 1.

Table 2 contains the results obtained by ashing the powdered dried whole egg in silica dishes without any previous treatment (1 and 2), with previous addition of 5 grams of 60-mesh alundum (3 and 4), with admixture of 5 cc. of 1 per cent magnesium acetate solution (5 and 6), and with addition of 5 grams of alundum and 5 cc. of 1 per cent magnesium acetate solution (7 and 8).

TABLE 2.

Results of ashing powdered dried eggs in silica dishes by various methods.

SAMPLE NO.	ADDITION	TOTAL HEATING TIME IN MUFFLE	ASH	COMMENTS
		<i>hours</i>	<i>per cent</i>	
1	7½	3.43	Marked tendency to creep up sides of dish. Difficult to burn off carbon. Necessary to leach with water several times. There seemed to be some unburned carbon.
2		8	3.48	
3	5 grams of Al ₂ O ₃	2½	3.30	Tendency to creep.
4		3	3.24	
5	5 cc. of 1% MgAc ₂	2	3.70	Very little creeping.
6	solution	2	3.68	
7	5 grams of Al ₂ O ₃ plus	1½	3.61	No spattering or creeping.
8	5 cc. of 1% MgAc ₂	1½	3.61	
	solution			Light gray ash in less than 1 hour. No leaching necessary.

This preliminary work indicated (1) that ashing liquid or dried egg without a hastener is a time-consuming, tedious operation, requiring several leachings with water; (2) that mixing alundum with the sample reduces the ashing time by more than half; (3) that the addition of magnesium acetate solution results in higher ash values than are obtained otherwise, reduces the ashing time, and prevents the creeping of the material up the sides of the dish during charring; and (4) that the previous addition of both magnesium acetate solution and alumina gives the highest ash value in the shortest time.

EFFECT OF ASHING ON DISH.

Attention was next turned to the effect of the ashing on the dish.

A weighed silica dish containing 10 grams of 60-mesh alundum was heated in a muffle at low red heat for 2 hours and then was removed and cooled. When the alundum was removed, it was found that the surface of the dish was slightly pitted and that some of the alundum had fused into the dish. The weight of the dish had decreased 10.0 mg.

To determine the effect of magnesium oxide on silica, 10 cc. of 1 per cent magnesium acetate solution was placed in a weighed silica dish and evaporated to dryness on a hot plate. The dish was heated in a muffle at low red heat until it reached constant weight; it was then cleaned with hot 10 per cent hydrochloric acid solution, dried, and weighed. The loss in weight was 3.0 mg.

A third silica dish containing 10 grams of alundum and 10 cc. of 1 per cent magnesium acetate solution was dried on a hot plate and ignited in a muffle at low red heat. The dish was then cooled, emptied, cleaned with hot 10 per cent hydrochloric acid solution, dried, and weighed. The loss in weight was 7.5 mg.

Since silica dishes are markedly attacked by the basic substances alumina and magnesium oxide, it was decided to investigate the feasibility of using dishes made of other materials. Nickel crucibles are fairly cheap and readily available, but it was found that they tarnish quickly and are difficult to clean.

The associate referee hesitated to attempt the ashing of eggs in platinum dishes, because the ash is acidic in character and contains much phosphorus. It seemed probable, however, that in the presence of a basic substance like magnesium oxide, the phosphorus would be combined as phosphate or pyrophosphate and would not attack the platinum. A determination on dried egg was made in a 17 gram platinum dish in which about 10 grams of alundum was mixed with 1 gram of dried powdered egg, and 10 cc. of 1 per cent magnesium acetate solution was added. The contents of the dish were dried on a hot plate and then ignited in a muffle at low red heat for 2 hours. The dish was then cooled, cleaned with hydrochloric acid, washed, dried, and weighed. The loss in three different trials was 0.3, 0.1, and 0.3 mg., respectively. There was no marked corrosion, but two of the dishes showed a discolored spot about 2 mm. in diameter.

Porcelain crucibles were next tested. Two crucibles of 40 cc. capacity lost 0.2 mg. each during ignition of 10 grams of alundum. Drying 10 cc. of 1 per cent magnesium acetate solution in porcelain crucibles, igniting at a low red heat, and cleaning with hydrochloric acid solution caused three crucibles to lose 0.4, 0.2, and 0.4 mg., respectively, in weight. No opportunity was presented to test the porcelain crucibles in an actual ash determination of liquid or dried eggs.

CONCLUSIONS.

The preliminary work reported led to the following conclusions:

- (1) The ashing of solid or liquid eggs without any previous treatment is impracticable and yields low results.
- (2) The addition of 60-mesh alumina (alundum) reduces the ashing time by three-fourths.
- (3) The addition of magnesium acetate solution causes higher ash results, indicating that in the absence of an excess of basic material some inorganic matter is lost by volatilization.
- (4) The addition of magnesium acetate solution prevents the creeping of the material up the sides of the ashing dish during the charring.
- (5) Silica dishes are markedly corroded by magnesium oxide, and particularly by granulated alumina, during ashing.
- (6) Platinum dishes do not appear to be attacked by egg ash in the presence of alumina and an excess of magnesium oxide.
- (7) Porcelain crucibles are not attacked by magnesium oxide or alumina on ignition at low red heat. They have not been tested in an actual determination of egg ash.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the method for ash in eggs worked out by the associate referee be subjected to collaborative study.
- (2) That the other methods recommended for the attention of the associate referee, but untouched this year, be studied next year.

REPORT ON THE DETECTION OF DECOMPOSITION
IN EGGS.

By H. I. MACOMBER (U. S. Food, Drug and Insecticide Administration,
New York, N. Y.), *Associate Referee*.

The method for the determination of the acidity of the fat, adopted at the last meeting as a tentative method, was submitted this year for collaborative study. Excellent results were obtained.

Samples of dried yolk and whole egg were sent to six collaborators to be analyzed for acidity of fat according to the method reported to the association and adopted last year.

The collaborators, all of whom are connected with the U. S. Food, Drug and Insecticide Administration, and the associate referee obtained the following results:

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 79.

Collaborative work on acidity of fat.(Results expressed as cc. of 0.05 *N* sodium ethylate per gram of fat.)

COLLABORATOR	WHOLE EGG	YOLK
J. I. Palmore Washington, D. C.	5.25	8.55
J. Calloway, Jr. Savannah, Ga.	5.35	8.55
V. E. Munsey Cincinnati, Ohio	4.93	8.78
C. A. Roach Chicago, Ill.	5.00	9.03
C. B. Stone Minneapolis, Minn.	5.38	8.37
D. B. Scott New York, N. Y.	4.95	8.80
H. R. Smith Baltimore, Md.	4.85	8.45
H. I. Macomber	4.98	8.52

The results given in the table are the averages of two or more determinations.

C. B. Stone reported that the end point in the titration is very difficult to determine. None of the other collaborators offered any comments or criticisms.

No further study was made this year of the method for the determination of acid-soluble phosphoric acid and the other methods for the detection of decomposition in eggs.

RECOMMENDATIONS¹.

It is recommended—

(1) That the method for the determination of the acidity of the fat, as described in last year's report, be adopted as an official method.

(2) That the method for the determination of acid-soluble phosphoric acid be given further study with the object of securing collaborative data.

(3) That a study be made of the methods for determining ammonia nitrogen and reducing substances as dextrose with the object of securing collaborative data.

No report on total solids in eggs and egg products was given by the associate referee.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 79.

REPORT ON FOOD PRESERVATIVES.

By WYATT W. RANDALL (State of Maryland Department of Health, Baltimore, Md.), *Referee*.

At the 1926 meeting the referee reported¹ that the work of the collaborating chemists showed:

(1) That the chloroform employed in the present official method for the determination of benzoate in ketchup extracts from the acidified sample small quantities of acid-reacting substance other than benzoic acid, whether the latter is present or not;

(2) That the sublimation process, applied to the dried residue extracted by chloroform from the acidified sample, tends to isolate from other acid-reacting material any benzoic acid that may have been present; and

(3) That the titration of the acid-reacting material, after the latter has been purified by sublimation, yields accurate results.

The referee recommended that before any change in the official method was requested, further study be given to the details of the processes of (a) filtration of the sample, (b) extraction by means of an immiscible solvent, and (c) sublimation of the dried extract; and that any suggested changes of method be tried out with products other than ketchup.

Accordingly, samples were prepared as described below, and instructions for the guidance of analysts were sent with them. The following analysts agreed to cooperate in this investigation: W. H. Schulze, State of Maryland Department of Health; V. B. Bonney and J. I. Palmore, U. S. Food, Drug and Insecticide Administration, Washington, D. C.; and W. C. Johnson and G. A. Dysterheft, Minnesota Dairy and Food Department, St. Paul, Minn. To each of them the thanks of the referee are due.

The instructions for the guidance of collaborators included a description of the eight samples issued; reasons for a renewed study of methods; grounds for the belief that the official method yields results indicating a higher percentage of benzoate than is actually present; a discussion of the probable superiority of ether over chloroform as an extracting solvent; a description of an improved form of extracting apparatus; etc. The details of these suggestions need not be given here.

The modified method, which was to be employed in comparison with the official method², varied in certain particulars from that discussed in the last report³; for example, ether was to be used in place of chloroform as an extracting solvent.

¹ *This Journal*, 1927, 10: 414.

² *Methods of Analysis*, A. O. A. C., 1925, 9, 10, 11: pp. 128-9.

³ *This Journal*, 1927, 10: 417.

SAMPLES DISTRIBUTED.

Each collaborator was provided with samples of the following preparations:

	TOTAL WEIGHT OR VOLUME	BENZOATE COMPONENT		
		Solution cc.	Content grams	Percentage
A. Tomato ketchup.....	2,000 grams*	10.67	1.600	0.080*
B. Tomato ketchup.....	2,000 grams*	8.00	1.200	0.060*
C. Orange-juice concentrate..	2,000 grams	13.33	2.000	0.100
D. Orange-juice concentrate..	2,000 grams	9.33	1.400	0.070
E. Cider (alcohol added)....	2,915 cc.	18.80	2.82	0.100*
F. Cider (alcohol added)....	2,915 cc.	13.14	1.97	0.070*
G. Crab-apple jelly.....	2,000 grams	8.00	1.200	0.060
H. Crab-apple jelly.....	2,000 grams	12.00	1.800	0.090

* Closely approximate.

According to their labels, none of the original commercial samples from which these samples were prepared contained any added preservative. The orange-juice concentrate and the crab-apple jelly were diluted and heated, as well as vigorously shaken, in order to secure thorough admixture of the added benzoate. The solution of sodium benzoate was prepared by dissolving 12.71 grams of pure benzoic acid in an excess of dilute sodium hydroxide solution and diluting to an exact volume of 100 cc.; 1 cc. of such a solution contains 0.150 gram of anhydrous benzoate.

ANALYTICAL RESULTS.

The collaborating analysts were requested to examine each of the eight samples submitted by both the official and the modified method and to run check determinations. In the case of the modified method, they were asked to report the weight of the crude ether extract after thorough drying, the loss in weight that this extract suffers through sublimation, the direct weight of the sublimate obtained, and the weight of benzoic acid calculated from the titration results. The number of determinations reported was as follows:

	OFFICIAL METHOD								MODIFIED METHOD							
	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H
Schulze.....	3	3	2	2	2	2	1	1	2	3	2	2	2	2	2	2
Bonney.....	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Palmore.....	4	2	2	2	2	2	2	2	2	4	2	2	2	2	2	2
Johnson-Dysterheft.....	1	..	1	2	2	4	..	2	2

Tables showing all these results in detail were constructed, but in view of the space that would be required for their exhibition it was

decided to print only the average percentages found by each analyst. In practically all cases there were but slight differences between the highest and the lowest percentage found for a given sample by a given analyst.

Averages of results reported by collaborating chemists.

		METHOD	SCHULZE	BONNEY	PALMORE	JOHNSON-DYSTERHEFT
	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Sample A	(0.080)	Official	0.0987	0.0790	0.0765	
		Modified	0.0805	0.0660	0.1540*	
Sample B	(0.060)	Official	0.0753	0.0630	0.0575	
		Modified	0.0592	0.0585	0.0726	
Sample C	(0.100)	Official	0.1140	0.0880	0.0870	
		Modified	0.0900	0.0715	0.0990	
Sample D	(0.070)	Official	0.0850	0.0615	0.0610	0.0820
		Modified	0.0670	0.0580	0.0385	0.0587
Sample E	(0.100)	Official	0.1047	0.0760	0.0930	
		Modified	0.0990	0.0690	0.0920	
Sample F	(0.070)	Official	0.0725	0.0695	0.0470	
		Modified	0.0640	0.0505	0.0545	0.0610
Sample G	(0.060?)†	Official	0.0910	0.0800	0.0500	0.0850
		Modified	0.0720	0.0615	0.0375	0.0760
Sample H	(0.090?)†	Official	0.0680	0.0460	0.0465	0.0515
		Modified	0.0355	0.0430	0.0390	0.0360

* Was a 200 cc. aliquot used in this case instead of one of 100 cc.? If so, the percentage becomes 0.0770.

† While these figures represent the exact amounts the referee intended to add and thought he had added, the results obtained by the several analysts seem to indicate that "G" actually contained 0.08 per cent, and "H" 0.04 per cent, of sodium benzoate. Unfortunately, it is now impossible to speak with certainty with respect to these two percentages.

COMMENTS BY COLLABORATORS.

W. H. Schulze.—All samples were filtered with the aid of the Büchner funnel and two filter papers. When the official method was followed, the salt-solution extract of the sample was made up to final volume *before* filtration; when the modified method was employed, the salt-solution extract was made up to final volume *after* filtration. In consequence, results by the official method are somewhat higher than they would have been if the more accurate procedure had been followed.

In all cases the Büchner-funnel method yielded very satisfactory filtrates. The time required for the filtration varied with the nature of the sample: ketchup required the most, cider the least, time.

Other advantages possessed by the proposed method over the official method are:

- (1) The elimination of extracted color before the final titration comes to be made, and hence a much sharper end point;
- (2) The fact that the weight lost by the ether extract upon sublimation closely approximates the direct weight of the sublimate and the weight of benzoic acid found by titration—provided the extract is thoroughly dried—enables the analyst to check titration results with fair accuracy;
- (3) The use of ether in place of chloroform for extraction avoids, in the majority of cases, the formation of emulsions that will not separate completely.

Johnson-Dysterheft.—When extracting substances such as ketchup, cider, fruit juices, etc., *i. e.*, substances that contain finely divided pulp, ether is not so good as chloroform.

¹ Somewhat condensed from the communication of Henry Hoffmann, Jr.

It is true that there is less danger of forming emulsions with ether than with chloroform, but in such substances as those mentioned the solubility of water in ether is a factor, because when the ether extract is evaporated to dryness, we always find that there is quite a large residue left in the dish. We notice that in the majority of cases the "weight of residue in dish" is larger when ether is used to extract, and also, with these substances, that the weight of the sublimate is less. Doubtless the large amount of gummy material extracted mechanically prevents the benzoic acid from subliming. Chloroform could not be used to transfer the residue to a sublimation dish, since it did not dissolve the gummy resins and, in order to insure the transfer of all the benzoic acid to the dish, we had to make use of a spatula and ether. We notice also that when ether is used to extract, the residue after sublimation has a very pronounced acidity—much higher than when use is made of chloroform. For this reason ether should not be used instead of chloroform if the residue is to be titrated directly, as in the official method.

Much trouble was experienced with emulsions in extracting the cider and the orange-juice concentrate—especially in the case of the cider. It sometimes required a day for the mixture to separate after shaking. More trouble was experienced with chloroform than with ether. In the case of cider, directions are given to heat over a water bath in order to remove the alcohol. In all cases this heating seems to divide the suspended matter more finely rather than to coagulate it.

We believe that an effort should be made to devise suitable means to precipitate the pulp completely. The filtrate, as now prepared, is deeply colored, especially after heating. It is probable that this filtrate is not a true solution, but that it holds some of the material in colloidal suspension. This suspension, on extraction, yields an emulsion.

If a means could be devised by which the benzoic acid could be quantitatively precipitated and then the precipitate purified in some way, as by extraction or sublimation, the pulp and interfering dissolved material would be eliminated.

DISCUSSION.

Until his work was completed, none of the analysts knew how much benzoate had been added to the respective samples.

The analysts usually found that enough color was extracted from the several samples to cause some difficulty in determining the end point of the titration. The process of sublimation served to reduce—often completely to remove—this difficulty. In general, it would appear that more color was extracted by chloroform than by ether.

The fact that the quantity of the crude residue left on evaporation of the immiscible solvent is usually greater when ether has been used instead of chloroform, does not, in the opinion of the referee, militate against the employment of ether, if *thorough drying* and sublimation are to be resorted to as a method of purification.

When the modified method was employed—and probably in those cases also where the official method was used—the crude extract contained material other than benzoic acid, sometimes in relatively large amount. When pains have been taken to dry this residue *thoroughly*, the loss of weight resulting from sublimation, and the direct weight of the sublimate itself, will each serve as a determination of the benzoic acid present, showing that sublimation serves an excellent purpose.

Clearly, the titration of a properly dried and sublimed product should be the most accurate method of all, since the benzoic acid would then be freed from more volatile and less volatile acid substances and, in most cases at least, from color as well.

The official method, in the opinion of the referee, usually yields results that appear better than they actually are, owing to the fact that acid substances other than benzoic acid are extracted by chloroform, while at the same time part of the benzoic acid is not extracted at all. Where the amount of such other acids is small, the two errors thus introduced may counterbalance one another. When much citric acid, for example, is present, the "benzoic acid found" may be quite appreciable, even when no benzoic acid is actually present in the sample. The modified method, if carefully conducted, will apparently—in the examination of a considerable variety of products—yield accurate results for benzoic acid, and will eliminate error due to the presence of citric acid.

Johnson and Dysterheft report serious difficulties in both the methods under consideration because of formation of emulsions. Naturally they would like to see a method devised in which "pulp" would be so completely removed that extraction could be conducted without such danger.

RECOMMENDATIONS¹

In view of the fact that the collaborators are not unanimous in approving the substitution of the modified method for the official method for the determination of sodium benzoate in food products, it would seem wise to postpone any such action until further study by a considerable number of collaborating analysts has demonstrated the superiority of one method over the other. Accordingly, it is recommended:

(1) That the search for a more accurate method for the determination of benzoic acid (or of sodium benzoate) in food products be continued.

(2) That in this work as many classes of food products as is possible be included in the study.

(3) That the application of the process of sublimation to the separation and purification of saccharin to be determined in food products be made the subject of study.

(4) That an effort be made to formulate a satisfactory method for the determination of hydrogen peroxide added to food products.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 79.

REPORT ON COLORING MATTERS IN FOODS.

By C. F. JABLONSKI¹ (U. S. Food, Drug and Insecticide Administration, New York, N. Y.), *Referee.*

A quantitative separation of amaranth from tartrazene was undertaken by the referee, but owing to lack of time it was impossible to complete the work.

A number of immiscible solvents and acid solutions were used to separate amaranth from tartrazene, but they did not prove successful. Precipitation in a quantitative manner brought out the fact that a number of salts of organic bases have the property of precipitating amaranth, but have no effect on tartrazene. The most effective of these salts were found to be naphthyl amine hydrochloride, benzidine hydrochloride, and pseudo cumidine sulfate.

As pseudo cumidine sulfate appeared to be the most effective, it was considered advisable to test the completeness of the reaction. Therefore, a 0.1 *N* solution of this salt in 0.5 *N* sulfuric acid was prepared, and a measured volume was added to a definite volume of standard amaranth solution. The results obtained were as follows:

0.5% SOLUTION OF AMARANTH	0.1 <i>N</i> PSEUDO CUMIDINE IN 0.5 <i>N</i> H ₂ SO ₄	AFTER 1 HOUR
cc.	cc.	
10	5	No precipitation
10	6	No precipitation
10	7	No precipitation
10	8	Slight precipitation
10	9	Slight precipitation
10	10	Considerable precipitation
10	15	Heavy precipitation
10	20	Heavy precipitation
10	25	Heavy precipitation

These results indicate the necessity of having a dye concentration of at least 50 mg. in 20 cc. of solution, as smaller quantities were not acted upon. Furthermore, the precipitation was by no means complete, as a small portion of the coloring matter remained in the supernatant liquid and could not be precipitated even by a further addition of the cumidine solution. Naturally, no additional work in that direction was considered.

Another method of precipitation and a gravimetric estimation of one of the components of amaranth was tried. Since amaranth is a product formed by coupling naphthionic acid with "R" salt, it was considered that if this dye was reduced, it might be possible to isolate the least soluble component by precipitation and to weigh it.

¹ Presented by D. B. Scott.

To test this theory 2.0 grams of amaranth (86.2 per cent purity) was dissolved in sufficient water to make 100 cc. From this solution the following quantities were measured out:

- (1)—1 cc. (0.0172 gram) + 9 cc. H₂O : 3 cc. 40% SnCl₂ : 5 cc. HCl : 20 cc. alcohol.
- (2)—2 cc. (0.0344 gram) + 8 cc. H₂O : 3 cc. 40% SnCl₂ : 5 cc. HCl : 20 cc. alcohol.
- (3)—3 cc. (0.0517 gram) + 7 cc. H₂O : 3 cc. 40% SnCl₂ : 5 cc. HCl : 20 cc. alcohol.
- (4)—4 cc. (0.0688 gram) + 6 cc. H₂O : 3 cc. 40% SnCl₂ : 5 cc. HCl : 20 cc. alcohol.
- (5)—5 cc. (0.0862 gram) + 5 cc. H₂O : 3 cc. 40% SnCl₂ : 5 cc. HCl : 20 cc. alcohol.
- (6)—6 cc. (0.1034 gram) + 4 cc. H₂O : 3 cc. 40% SnCl₂ : 5 cc. HCl : 20 cc. alcohol.

The results obtained were as follows:

No. 1 and 2—no precipitate after 48 hours.

No. 3—precipitate within 24 hours.

No. 4—precipitate after 2 hours.

No. 5—heavy precipitate after 2 hours.

No. 6—heavy precipitate immediately.

After standing 24 hours the precipitate was transferred to a weighed Gooch crucible and washed with a solution containing 10 cc. of concentrated hydrochloric acid to every 100 cc. of alcohol. After drying at 100°C. the following weights were obtained:

		THEORY	RECOVERED
	gram	gram	per cent
(3)	0 0162	0 01987	81 5
(4)	0 0227	0.02649	85.7
(5)	0 0243	0.03311	73 4
(6)	0 0274	0.03974	70.0

These results were not encouraging, as again it was found to be essential to have at least 50 mg. of amaranth present; furthermore, the quantitative yield was by no means satisfactory.

A method was then considered to reduce the coloring matter, and diazotize and couple the reduced product, with a component that would form dyes of a solubility ratio different from those of amaranth and tartrazene. A separation of the dyes was attempted by extracting with immiscible solvents and washing these solvents with suitable acid solutions. β -naphthol was found to be the most suitable component to form dyes of unlike solubility ratio. To ascertain the feasibility of this theory a number of qualitative experiments were undertaken, but before going into detail it may be well to mention the behavior of amaranth and tartrazene towards zinc dust. When reduced with zinc powder, either in neutral, slightly ammoniacal, or acetic acid solution, a dilute aqueous solution of tartrazene develops a deep wine-red color on standing. A solution of amaranth under the same condition becomes green or orange brown.

Amaranth—S & J No. 107, C. I. No. 184—when reduced reverts to its components, namely, naphthionic acid and “R” salt; tartrazene—S & J No. 94, C. I. No. 640—however, splits into sulfanilic acid and phenylhydrazine sulfonic acid, which in turn is converted into sulfanilic acid by activated reducing agents, as well as into other organic substances which do not enter into a subsequent dye combination. When naphthionic acid is coupled with β -naphthol, Fast Red A—S & J No. 102, C. I. No. 176—is formed. Coupling sulfanilic acid with β -naphthol, Orange II—S & J No. 86, C. I. No. 151—is produced. Since these dyes possess an unlike solubility ratio, it was considered feasible to separate them. For practical purposes it was proposed to prove the presence and subsequently the amount of fast red A.

EXPERIMENTAL WORK.

To establish the sensitivity of the method, a number of solutions of mixtures of amaranth and tartrazene were prepared. They consisted of—

AMARANTH	TARTRAZENE
gram	gram
(a)—0.010	0.120
(b)—0.006	0.140
(c)—0.002	0.160
(d)—0.001	0.160
(e)—0.0002	0.160
(1)—0.200	0.002
(2)—0.200	0.010

In each case water was added to make the volume 25 cc. Then 2 cc. of concentrated hydrochloric acid and 3 cc. of 40 per cent stannous chloride in hydrochloric acid were added, and the solution was gently heated until decolorized. After cooling, 5 cc. of concentrated ammonium hydroxide to a slightly alkaline reaction and 25 cc. of 95 per cent alcohol were added. The resultant mixtures were transferred to flasks and centrifuged to obtain a clean separation, and the supernatant clear liquid was filtered into a small beaker (150 cc.). The magma in the flasks was reextracted with two 25 cc. portions of a mixture consisting of 200 cc. of alcohol, 30 cc. of ammonia, and sufficient water to make up to 300 cc. and centrifuged as before, the liquid portion being filtered into the beaker. The contents of the beaker were evaporated to dryness on the steam bath, redissolved with 10 cc. of water, and 5 cc. of concentrated hydrochloric acid was added. The beaker was placed in ice water at about 5°C., and 3 cc. of 10 per cent nitrite solution was added. The beaker was kept in the bath with occasional stirrings for 2 hours. In

another flask 12 cc. of 1 per cent β -naphthol (in dilute alcohol) and 50 cc. of 0.5 *N* sodium carbonate solution were mixed and cooled to 15°C. After the elapsed time the diazo solution was poured slowly into the alkaline β -naphthol, mixed thoroughly, and kept at room temperature (25°C.) for 3 hours. The resultant product was acidified with 25 cc. of 99.5 per cent acetic acid and extracted with three 50 cc. portions of amyl alcohol in rotation. The aqueous part was discarded, and the amyl alcohol extract was washed with 50 cc. portions of 1/512 *N* hydrochloric acid by passing them successively through the three separators. When large amounts of fast red A were present, three or four washings were found sufficient, as orange II washed out with straw yellow color and was discarded. In every instance there was a sharp differentiation between the orange yellow and the deep red shade of fast red A. After fractionating off the orange, the amyl alcohol extract was diluted with 40 cc. portions of petroleum ether, and the red dye was washed out with water, concentrated, and estimated. When the quantity of fast red A was small, however, it was necessary to dilute each amyl alcohol fraction with 40 cc. of petroleum ether and continue washing until all yellow color was removed. There was always a phase noted when no dye was extracted. When this stage was reached, the immiscible solvent was extracted with water until freed from the red coloring matter, which was then concentrated and estimated. This method made it possible to prove the presence of minute quantities of fast red A, as two parts in 1600 were readily detected. Since there is no chemical method known to detect amaranth in tartrazene, or tartrazene in amaranth, the importance of this problem is readily seen. Owing to the fact that experiments a, b, c, d, and e contained only small quantities of amaranth (less than 9 mg. maximum), it was not considered advisable to determine the resultant fast red A volumetrically, but to resort to a colorimetric estimation. The results obtained were found quite concordant with the original quantities taken.

In the case of experiments 1 and 2, however, which contained 0.2 gram each of amaranth, the resultant fast red A was estimated with titanium trichloride. The titer for both was exactly 70 per cent of the theory, but the referee believes that an error in manipulation during the separation was accountable for the discrepancy. Lack of time prevented further study of the subject, but it is important that additional experiments be undertaken to obtain a quantitative method for the separation and estimation of amaranth in mixtures.

RECOMMENDATIONS¹.

It is recommended—

- (1) That further study be devoted to the method described in this

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 80.

report in regard to quantitative separation and estimation of amaranth and tartrazene, and that more experimental data be collected.

(2) That work be undertaken to obtain a chemical method for separation and quantitative estimation of fast green FCF (recently adopted) from light green SF yellowish and guinea green B and other permitted dyes.

REPORT ON METALS IN FOODS.

By W. F. CLARKE (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

For collaborative study this year three modifications of the Gutzeit method and one feature of a fourth modification were chosen. These methods have been designated, respectively, the Western District¹, the Baltimore Station¹, the New York Station¹, and the Heidenhain² modifications. Only the temperature control feature of the Heidenhain modification was studied, and in this connection the Western District modification was followed otherwise.

PROCEDURE.

WET COMBUSTION.

In all the work the destruction of organic matter was effected by the usual acid combustion, except that (1) the final traces of resistant organic matter were oxidized by boiling the sulfuric acid actively for 10 minutes, and (2) the complete expulsion of the oxides of nitrogen was effected by the use of ammonium oxalate.

REDUCTION AND EVOLUTION.

The conditions under which reduction to trivalent arsenic is effected constitute the essential difference in these modifications.

When the Western District modification was used, for one set of determinations the aliquot of the digested sample was placed in a 2 ounce bottle, the volume was adjusted to 30 cc. by the addition of water, and 5 cc. of concentrated sulfuric acid was added; for the other set a 50 cc. Erlenmeyer flask was substituted for the 2 ounce bottle and 5 cc. of concentrated hydrochloric acid was used in place of sulfuric. In all determinations in which this modification was used 5 cc. of potassium iodide and 4 drops of stannous chloride solution (40 grams of stannous chloride in a sufficient quantity of concentrated hydrochloric acid to make 100 cc.) were added. After being mixed, the solutions were cooled to 17°-19°C. Evolution tubes were substituted for the bottles and prepared according to the A. O. A. C. official method; for the flasks 5 per cent lead acetate glass wool was substituted for cotton, and 2 mm. of

¹ Of the Food, Drug and Insecticide Administration, U. S. Dept. Agriculture.

² *This Journal*, 1928, 11: 107.

5 per cent mercuric bromide papers were prepared by the method of Kemmerer and Schrenk¹. For the arsine generation, 15 grams of sensitized² stick zinc was added to each of the bottles and to each of the flasks there was added 10 grams of granulated zinc (60 mesh). In both cases the reaction was allowed to run 1½ hours.

HEIDENHAIN TEMPERATURE CONTROL.

The Western District method was followed with one exception: during the evolution of arsine the generators were submerged in a five gallon water bath in such a way that the water reached to within ¼ inch of the top of the tube containing the paper strips.

In the study of the Baltimore Station modification for the generators, only the 2 ounce bottles were used, the aliquot of the digested sample was adjusted to 30 cc. as above, and 5 cc. of concentrated sulfuric acid and 5 cc. of potassium iodide were added. The contents of the bottles were heated in a bath to 90°C., and 3 drops of stannous chloride was added after the gas was turned off. The bottles were shaken and then allowed to stand 5 minutes. The temperature was reduced quickly to 17°-19°C. The evolution tubes were prepared as in the official method, 15 grams of sensitized stick zinc was added to the bottles, the tubes were connected, and the action was allowed to run 1 hour thus and 30 minutes longer after removal from the bath.

When the New York Station modification was followed, the aliquots taken were such that readings would be obtained around the 80-90 mmg. range. In place of sulfuric acid, 5 cc. of concentrated hydrochloric acid was used. From this point the Baltimore Station procedure was followed, except (1) the generators were heated to incipient boiling on a hot plate; (2) after removal from the hot plate, 4 drops of stannous chloride was added; (3) the generators were kept in cracked ice 45 minutes; (4) about 7.5 grams of unsensitized zinc (washed in dilute hydrochloric acid) and 7.5 grams of previously used zinc (hydrochloric acid washed) were used (pitted zinc rejected); and (5) during the first 30 minutes of the arsine evolution the bottles were kept in ice, for the next hour in the open air, and finally on a hot plate where they were heated to boiling.

READINGS.

The strips were read in the usual way, then the areas of the stains were measured and compared.

RESULTS AND COMMENTS.

Tables 1, 2, and 3 give the results obtained. The referee obtained erratic results, his figures for standards being such that comparisons were impossible; lack of time prevented repetition.

¹ *Ind. Eng. Chem.*, 1926, 18: 707.

² *This Journal*, 1927, 10: 427.

TABLE 1.

Results obtained by Leonard Feldstein, Denver, Colo.(All results are expressed as As_2O_3 .)

PART I.—WESTERN DISTRICT METHOD.

SOLUTION	DIGESTED WITH ORGANIC MATTER							
	In Erlenmeyer flask with glass wool		In bottles with cotton		In Erlenmeyer flask with glass wool		In bottles with cotton	
ml.	microgm.	area*	microgm.	area*	microgm.	area*	microgm.	area*
10 (A)†	30	534	30	522	30 *	624	29	522
10 (B)†	27	504	28	522	29	642	30	567
10 (A)	30	582
20†	20	490	20	384	20	438	20	414
25†	25	494	25	438	25	455	25	510
30†	30	558	30	504	30	696	30	552
35†	35	558	35	540	35	656	35	730

HAIDENHAIN CONSTANT TEMPERATURE BATH.

10 (A)†	30	588	30	672	30	582	31	545
10 (B)†	28	599	29	480	32	480	29	511
20†	20	360	20	324	20	432	20	492
25†	25	570	25	504	25	336	25	555
30†	30	576	30	485	30	540	30	400
35†	35	546	35	585	35	630	35	833

PART II.—BALTIMORE STATION METHOD.

SOLUTION	DIGESTED WITH ORGANIC MATTER			
ml.	microgm.	area*	microgm.	area*
10 (A)†	31-31	588-630
10 (B)†	28-30	580-516
20†	20	396	20	450
25†	25	522	25	444
30†	30	582	30	544
35†	35	612	35	600

PART III.—NEW YORK STATION METHOD.

30 (A)†	88-90	781-868
30 (B)†	76	698
75†	75	750	75	798
80†	80	765	80	804
85†	85	765	85	820
90†	90	888	90	972
95†	95	915	95	900

* Area given in 4096 parts of sq. in.

† Solution A.—10 ml. of unknown No. 1 diluted to 1 liter; Solution B.—10 ml. of unknown No. 2 diluted to 1 liter.

‡ The quantities 20, 25, 30, 35, 75, 80, 85, 90, 95 ml. correspond, respectively, to the same quantities mmg. As_2O_3 , and constitute the standards.

Feldstein prefers the Western District modification because of its simplicity, and from previous experience he thinks the wet combustion is less subject to bumping if the initial charge of nitric acid is 50 cc. Smith states that there is no loss of arsenic in the acid digestion

TABLE 2.

Results obtained by H. R. Smith, Baltimore, Md.(All results are expressed as As_2O_3).

SAMPLE*	QUANTITY TAKEN	BALTIMORE METHOD	NEW YORK METHOD
1	10 ml.	3.3 mg. As_2O_3	2.1 mg. As_2O_3
2	10 ml.	2.9-2.6	2.1-2.6
A	4 mg. As_2O_3	3.6-4.0	3.3-4.1-4.0
B	9 mg. As_2O_3	9.2	8.6-7.9
C	10 mg. As_2O_3	10.0	10.0
D	Blank	None	Slight amount

* 1 = 10 ml. sol. 1 + 50 gm. sugar.

2 = 10 ml. sol. 2 + 50 gm. sugar.

A = 4 mg. As_2O_3 + 50 gm. sugar.B = 9 mg. As_2O_3 + 50 gm. sugar.C = 10 mg. As_2O_3 without sugar

D = Blank on 50 gm. sugar + reagents.

All samples were digested and made to 1000 ml. Arsenic was determined on suitable aliquots by the Baltimore Station method, H_2SO_4 being used. Satisfactory stains were obtained and results are expressed in terms of As_2O_3 in the entire sample taken.

Other aliquots were taken and treated by the N. Y. Station method: 5 ml. conc. HCl and cooling to below $5^\circ C$. The stains obtained were not so satisfactory as in the case of the more familiar H_2SO_4 evolution.

NOTE.—As determined by the volumetric bromate method 10 ml. of the unknown solutions contained—

No. 1 = 3.09 mg. As_2O_3 .No. 2 = 2.89 mg. As_2O_3 .

TABLE 3.

Results obtained by W. C. Taber, San Francisco, Calif.(All results are expressed as As_2O_3 .)

SAMPLE SOLUTION	METHOD 1* H_2SO_4 , lead acetate cotton, 5% bromide paper	METHOD 2 HCl, lead acetate glass wool granulated zinc, 5% bromide paper	METHOD 3 Stick zinc with 14% bromide paper	METHOD 4 Heidenhain temperature control $22^\circ C$, 2.5% bromide paper and granulated zinc	METHOD 5 Same as Method 4 with stick zinc
ml.	microgram	microgram	microgram	microgram	microgram
10 (A)†	33	24	34 29 31	33	29
10 (B)†	28	22.5	27 25 24	30	25
Blank	None	Trace	None		

* All the methods were variations (as noted) of the Western District modification. All readings were made by comparison of measured areas of stains and standards.

† Solution A.—10 ml. of unknown No. 1 diluted to 1 liter;

Solution B.—10 ml. of unknown No. 2 diluted to 1 liter.

and that results by the Gutzeit methods are fairly satisfactory. From general observation he concludes that each analyst obtains the best results by use of the method with which he is most familiar.

Taber introduced certain minor changes, which are noted. He states that with the zinc obtainable, the use of 5 per cent bromide paper effects stains shorter than can be read accurately. He mentions that experience in the San Francisco laboratory indicates (1) that granulated zinc is not preferable to stick zinc, though theory might indicate it to be;

and (2) that potassium iodide and stannous chloride may be omitted where granulated zinc is used. Taber considers the area measurement method of reading to be a helpful addition.

The results are reported separately because so many variations were introduced that tabulation was impracticable.

The two solutions sent out contained arsenic corresponding to the following figures: No. 1 contained 3.0 mg. As_2O_3 per 10 ml.; No. 2 contained 2.7 mg. As_2O_3 per 10 ml. The collaborators were directed to take 10 ml. of these solutions, and after treatment to dilute to 1 liter and to use aliquots, as shown in the work reported by Feldstein and Taber. The same principle was followed by Smith, but the aliquots used were not reported, the calculations being referred to the original solutions.

CONCLUSIONS.

Although the results obtained are very good in some instances, general experience has made it plain that not one of the various modifications of the Gutzeit method can be used by the average analyst with the assurance or even probability that his results will be accurate unless he attains considerable experience in its use. A volumetric method capable of detecting small quantities of arsenic, subject to better control and simpler than the Gutzeit method, is surely to be desired.

RECOMMENDATIONS¹.

It is recommended—

(1) That the methods and modifications specified in this report be studied with a view to proposing one or more of them as alternative with the official method and that a suitable volumetric method be sought.

(2) That tentative method No. I, Chapter VIII, p. 90, *Methods of Analysis*, be so modified as to be applicable to foods and that the resulting modification and the thiocyanate modification of this method be studied collaboratively.

No report on zinc in dried eggs was given by the associate referee.

REPORT ON FRUITS AND FRUIT PRODUCTS.

By H. J. WICHMANN (U. S. Food, Drug and Insecticide Administration, San Francisco, Calif.), *Referee*.

Two associate referees were appointed by the association to carry on the work on fruit products. A report was received from Doris H. Tilden on Ash in Fruit Products, but E. K. Nelson, who has been endeavoring to devise a practical method for the determination of malic acid that

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 80.

does not depend upon its optical properties, had not completed his work when this report was written.

The referee reserved for himself the determination of solids in sucrose solutions containing organic acid, but he found it impossible to do the work he had planned.

The referee reports with much satisfaction that during the past year one troublesome source of error in the determination of the organic acids of fruits was apparently solved. Hartmann and Hillig have published a paper on the application of the Stahr reaction to the accurate determination of citric acid¹. They have apparently succeeded in determining the nature of the errors in the tentative method² and restricted them. The application of a solubility factor enables the analyst to obtain satisfactory results for small quantities of citric acid. This was always the weak point of the method. The referee has frequently noted in fruit analyses that the determined acids did not check the total acidity and that the percentage of citric acid was often ridiculously low. Even before the publication of the Hartmann-Hillig article the referee often speculated on the reasons for the unsatisfactory results for citric acid, thinking the low results might be due to solubility factors or to incomplete reactions. At his suggestion Analyst Tilden had plotted weights of pentabromacetone obtained against known varying amounts of citric acid, keeping the volume of the reaction mixture reasonably constant. She found that the points fell practically on a straight line parallel to and below the straight line representing the conversion factor 0.424. This would indicate that the principal error was a constant error, such as might be due to the solubility of the pentabromacetone. Results obtained by Hartmann and Hillig show practically the same thing. Solubility causes an enormous percentage error in the citric acid determination if the quantity of citric acid to be determined is small unless a correction is applied. This is just what these authors have done. It is recommended that the next Associate Referee on Fruit Acids test this modified citric acid method collaboratively to demonstrate its fitness for adoption as a tentative method in place of the present one.

The Associate Referee on Ash in Fruit Products devoted as much time as could be spared from her regular duties to the study of ash analysis. The collaborative study of the calcium and magnesium methods recommended by the association at its last meeting was not carried out, principally because it was desired to fit the determination of manganese into this scheme of ash analysis. This has now been done, at least so far as small quantities of this element, such as are apt to be encountered in plants, are concerned.

¹ *This Journal*, 1927, 10: 264.

² *Methods of Analysis*, A. O. A. C., 1925, 215.

The official Lindo-Gladding method for potash, a most important constituent of fruit ashes, has also been tested for small quantities and a minor modification found desirable. The way now seems clear to do collaborative work next year on the determination of potash, calcium, magnesium, and manganese, the four most important bases of fruit ashes.

Tilden also made a report on the determination of chlorine in the presence of organic matter to the General Referee on Plants. It is also of interest to the Referee on Fruits and Fruit Products. The associate referee found that the determination of chlorine in the ash was worthless. The direct gravimetric method on fruit juices was also generally unsatisfactory because of the colloidal character of such products. Direct volumetric methods were also not considered promising, except in special cases. The point of interest in the report is the fact that chlorides can be fixed during ashing provided there is present a sufficient excess of sodium carbonate. It is recommended that the official methods¹ for chlorine in fruit products be described merely as chlorine in the ash, and that as soon as the associate referee's method can be tested and perhaps adopted tentatively the present method be dropped.

The referee wishes to call the association's attention to the definitions for pectin substances recommended by the Committee on Nomenclature of Pectin of the Agriculture Food Division of the American Chemical Society. These definitions and a statement of the committee were published in the Proceedings in the May number of the *Journal of the American Chemical Society*. The referee recommends that this report be considered by this association and that the definitions be endorsed².

REPORT ON FRUIT ACIDS.

By E. K. NELSON (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The most notable development of the year in the line of analytical problems connected with the determination of fruit acids was the study of the Stahre reaction for the determination of citric acid, published by Hartmann and Hillig³.

Further work by these authors⁴ shows that acetone is a splendid solvent for extracting citric, tartaric, and malic acids in relatively pure form from sugar. As has been reported previously⁵ by the associate referee, the determination of malic acid as fumaric acid can be carried out satisfactorily only when the acids are free from foreign substances, a condi-

¹ *Methods of Analysis*, A. O. A. C., 1925, 211.

² For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 80.

³ *This Journal*, 1927, 10: 284.

⁴ Unpublished.

⁵ *This Journal*, 1927, 10: 432.

tion which he was unable to meet in the experiments in which the acids were separated by means of barium or ether. It is possible that better success may be had in the use of acetone, as proposed by Hartmann and Hillig.

It was found that malic acid can be converted into fumaric acid and that the fumaric acid can be weighed as such or as mercury fumarate. It is recommended¹ that the work on the extraction and gravimetric determination of malic acid be continued, with a view to obtaining the acid in a sufficient state of purity to apply the fumaric acid method.

REPORT ON ASH IN FRUIT PRODUCTS.

By DORIS H. TILDEN (U. S. Food, Drug and Insecticide Administration, San Francisco, Calif.), *Associate Referee*.

At the last meeting the Associate Referee on Ash in Fruit Products reported on new methods for the determination of calcium and magnesium in plant ashes². The association recommended that collaborative work be done on these methods, and that efforts be continued in devising methods for determining iron, aluminum, manganese, and chlorine in plant products, particularly fruits. The present associate referee has determined the conditions necessary for the determination of chlorine in carbonaceous products and has made a separate report to the Referee on Plants. Collaborative work on calcium and magnesium, however, was postponed for a year to enable the referee to study methods for the determination of manganese and potash in the amounts found in fruits before the collaborative samples were sent out. Because these elements are important in the analysis of the ash of fruit products it was desired to recommend methods for their determination at the same time that samples were sent to collaborators.

MANGANESE EXPERIMENTS.

The colorimetric periodate method for the determination of manganese³ was found to be quite satisfactory for small quantities such as are generally found in plant products⁴. The referee desired, however, to avoid a separate ashing and to combine a manganese method with the methods for calcium and magnesium in plant ashes reported last year. At that time it was indicated that manganese was completely removed from ash solutions as manganese dioxide by the sodium acetate, acetic acid, bromine water method of separation and that no calcium or magnesium was lost in the process. Since the calcium and magnesium methods

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 81.

² *This Journal*, 1927, 10: 433.

³ *J. Am. Chem. Soc.*, 1917, 39: 2366; *Methods of Analysis*, A. O. A. C., 1925, 42

⁴ *Ind. Eng. Chem.*, 1926, 18: 172.

require a previous removal of manganese, the possibility of using this precipitate in the quantitative determination of the latter element was obvious. Gravimetric methods involving precipitation of the manganese as manganese dioxide and ignition to manganomanganic oxide are said to yield only approximate results owing to the possible occlusion of calcium and the fact that the composition of the ignited manganomanganic oxide is not always constant¹. A colorimetric method should be free from such objections. Therefore an attempt was made to combine the separation of manganese as manganese dioxide and its determination both gravimetrically and colorimetrically.

For the experimental work a known solution of a manganous salt was prepared by reducing a known volume of standard 0.1 *N* potassium permanganate with sulfur dioxide. The excess sulfur dioxide was boiled off, and the solution was cooled and diluted to a definite volume with distilled water. Aliquots of this solution were treated according to the method recommended last year, and the precipitated manganese dioxide was collected on a filter paper and washed thoroughly with hot water. When a gravimetric determination was desired the filter and charge were placed in a weighed crucible, ignited gradually to dull redness, and held at that temperature for 10–15 minutes. After cooling, the manganese was weighed as manganomanganic oxide. For the colorimetric determination the manganese dioxide was washed from the filter with hot water and dissolved in 50 per cent sulfuric acid and 50 per cent nitric acid. The two acids, used separately, are necessary to secure complete solution. After adding 5 cc. of concentrated sulfuric acid, the solution was boiled to expel nitric oxide fumes. From this point the official colorimetric periodate method was followed. The results obtained are given in Table 1.

TABLE 1.

Comparison of the gravimetric and colorimetric methods for the determination of manganese.

MANGANESE PRESENT CALCULATED AS Mn_2O_4		MANGANESE RECOVERED CALCULATED AS Mn_2O_4	
mg.		mg.	
	Gravimetric Determination		
14.9		15.2	
7.4		7.4	
3.7		3.9	
	Colorimetric Periodate Determination		
7.47		7.55	
7.47		7.23	
7.47		7.45	
2.98		2.82	
2.98		2.80	
1.49		1.47	
1.49		1.51	

¹ Treadwell and Hall. *Analytical Chemistry*, Vol. 2, p. 108.

These two methods for manganese, combined with the separation from calcium and magnesium, appear to be promising provided the element is not present in amounts exceeding 10 mg., which is hardly probable in the quantities of plant products usually taken for analysis. The referee did not have sufficient time to study the gravimetric method thoroughly for larger amounts of manganese or when it was found in the presence of considerable quantities of alkaline earths. No doubt the colorimetric method can be used to best advantage when small quantities of manganese are present, owing to the deep color developed with larger quantities. A little familiarity with the method produces rapid and satisfactory results.

POTASH EXPERIMENTS.

The official Lindo-Gladding method for potash in fertilizers¹ was designed primarily for considerably larger quantities of potassium than those usually found in plant products, when a matter of a few milligrams is often a decisive factor in determining the amount of plant material in an unknown mixture. For fertilizer work where comparatively large quantities of potassium are present, Hazen² recommends two wash solutions for the potassium platonic chloride—90 per cent alcohol in the initial treatment and 80 per cent alcohol in the final washings to minimize the solubility of potassium platonic chloride—which suggested to the writer that for small quantities of potassium 90 per cent alcohol for both washes might be advantageous. In order to try out this idea, two experiments were devised. In one the composition of mustard seed (relatively low in calcium and magnesium and high in phosphorus) was approximated, and in the second the composition of fruit products containing calcium, magnesium, and phosphorus in about equal quantities was imitated. The results obtained are given in Table 2.

TABLE 2.

Effect of strength of alcohol on wash solutions in Lindo-Gladding method for potash.

K ₂ O PRESENT mg.	K ₂ O RECOVERED	
	80 % alcohol washes	90 % alcohol washes
Ash representing mustard seed		
6.31	6.06	6.33
63.17	61.34	62.33
63.17 + 0.3 gram additional Na ₂ HPO ₄ 12H ₂ O		62.95
6.31 + " " "		7.43
Ash representing fruit		
6.31	6.04	6.27
63.17	58.61	63.57
		62.79

¹ *Methods of Analysis*, A. O. A. C., 1925, 13.

² *This Journal*, 1922, 5: 456.

The 80 per cent wash solution tends to produce low results, probably due to solubility of the potassium platonic chloride. If 90 per cent alcohol wash solutions are employed, more washing is required, usually 200-250 cc. being necessary. The referee would suggest that after washing, drying, and weighing of the precipitate, its purity be tested by washing it again with several 10 cc. portions of 90 per cent alcohol, drying and weighing until a constant weight of platonic chloride is obtained. In the referee's opinion this modification of the Lindo-Gladding method will produce quite accurate and satisfactory results for potash in plant ashes.

SUMMARY.

(1) The gravimetric method for the determination of manganese in the ash of plant material, as well as the colorimetric periodate method, has given satisfactory results for small quantities.

(2) The sodium acetate-bromine separation of manganese prior to the calcium determination specified in the proposed methods for calcium and magnesium gives a precipitate that may be used for a quantitative manganese determination by either the gravimetric or the colorimetric method.

(3) The present procedure in the official Lindo-Gladding method of washing potassium chloroplatinate with 80 per cent alcohol probably causes slight errors due to solubility.

(4) Small quantities of potassium platonic chloride appear to be soluble in an 80 per cent alcohol wash solution, and not in a 90 per cent wash solution.

(5) A modification of the Lindo-Gladding method for potash specifying a complete wash with a 90 per cent solution gives very satisfactory results.

RECOMMENDATIONS¹.

It is recommended—

(1) That a method for the determination of larger quantities of manganese be studied and that collaborative work be done on the determination of the major bases in plant ashes—potash, calcium, magnesium, and manganese.

(2) That methods for the determination of iron and aluminum in plant ashes be undertaken.

No report on canned foods was given by the referee.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 81.

ADDRESS BY DR. WILEY.

MR. PRESIDENT, MEMBERS OF THE ASSOCIATION,
LADIES, AND GENTLEMEN:

You will excuse me, I am sure, for a few reminiscences on an occasion like this. They are based upon what I have learned this year. I am still going to school. Last year I graduated from an investment banking school; this year I have taken a chemical course. I may know something about chemistry before I die.

I am carried back to the very beginning of this science as we know it now. When I became head of the Chemistry Department of Purdue University, in 1874, little was known compared to what we now know about agricultural chemistry. There were three outstanding men in this country at that time in this field—Eugene W. Hilgard, Samuel W. Johnson, and Francis H. Storer of Harvard University. Other persons knew something about agricultural chemistry, but these men were the most prominent. Because their principles were fundamentally correct, their fame will always endure. If they could have looked forward to this time and could have seen the great progress made in their science they would hardly have believed it possible.

At the beginning of my active career as a chemist in Butler College in 1873, I inaugurated a new method of teaching chemistry in Indiana. I had previously learned this science from a text book and from the illustrated lectures of Professor J. W. Scott of Hanover College, but I had never been put to work on a laboratory task until my later years in college, when I assisted the professor of chemistry to some extent with the younger classes. The new system, which I used first in Indiana, consisted in putting beginners at experimental laboratory tasks. We began the first day doing something of a chemical character.

At that time Professor Campbell of Wabash College was much in the public eye, because he was the first man to propose the holding of a Centennial Exposition at Philadelphia on the 100th anniversary of the signing of the Declaration of Independence. Later, because he had suggested it, he was made secretary of that great organization which at that time was preparing for this exposition. I conceived the idea that it might be interesting, and it certainly would be informative, if some exhibit of chemical industry could be made at the Centennial Exposition. I got in touch with Professor Campbell, who was a very dear friend of mine, and he heartily approved the plan. I immediately began, therefore, to have my students prepare samples of inorganic chemicals. We

hadn't reached the age of organic chemistry, which was then just beginning. We had had the principles of chemistry only up to that point. None of the pupils knew about chemistry; I didn't know much, but they never discovered that. By the time the Exposition opened, we had prepared 100 samples of inorganic chemicals, all put up in crystalline form in an attractive way, and these were exhibited in 1876 at the great centennial celebration of our birth as a nation. That was the first college exhibit of chemistry ever made in this country. I wish now that I had saved those samples, but I didn't consider them of any particular value. I didn't take them home with me and suppose they were destroyed when the exhibition was dismantled. It is interesting to recall them at this time in connection with some other things that I want to say about exhibits of chemical proficiency.

I attended the wonderful chemical exhibit of the products of chemical engineering at the Grand Central Palace in New York a few weeks ago. On three immense floors of that great building all the progress that had been made in chemical engineering was demonstrated. It was almost overwhelming in its effect on me, at least, to see what had been accomplished in chemical science. Of course, a great part of it related to agricultural chemistry. Connected with this exhibit was a school, which I attended. So I got my name in the paper again as going to school. This was a class of advanced but under-graduate students of chemical engineering. The members came from the Universities of Pennsylvania, Columbia, Princeton, Yale, Harvard, and other nearby colleges. There were about 125 of them altogether. They met on the first day of the exhibit and I met with them, and to me that was the most interesting feature of the exhibit. These young men, who were proposing to give their lives to practical chemical science, were assembled to study the products they were intending to make or help to make when they came into active life. They had every facility given them. The doors were not open to the public until 12 noon, but these young men met at 9 o'clock in the morning. They had addresses or lectures by capable chemical engineers, and then went privately with their professors and studied the products and mechanical apparatus before the public was admitted. This plan provided ample opportunity to see what was going on under competent instruction. I went around with those young men every day and learned perhaps as much as they did from their professors. I gave two or three of the lectures myself—not like this one, unprepared. So that was a wonderful exhibit. I cannot describe its importance to me.

There were one or two other things about it, more or less relating to agriculture, to which I should like to call your attention. I saw there, in great quantities, blocks of dry ice (frozen carbon dioxide) about one

foot square, and the literature connected with it told what had been accomplished by it, particularly in the transportation of perishable agricultural products. I didn't question the sincerity of those statements, but they were so remarkable that if anybody had shown me such an advertisement I should have thought it too exaggerated. It was stated that a block of dry ice, such as was exhibited, would not disappear for many, many days, and that by placing one block in a car or a box of perishable agricultural products it could be sent across the country in a frozen condition and would be much colder when it reached that side than when it left this side. In the hand this material really burns. Its temperature, far below zero, is even colder than the Arctics. A piece of it thrown into a jar of water gives off bubbles of gas, quite rapidly at first, but slower later on, because it freezes ice around itself and so prevents its more rapid evaporation into the air. Dry ice is one of the great discoveries and inventions of chemical engineering, and it means a great deal to agricultural prosperity. It was one of the most remarkable things I saw at the exhibit.

Now, what does this all mean? It not only means that through our efforts in agricultural chemistry we have brought order out of chaos, but it assures us that in the transportation of perishable agricultural products we have a method of refrigeration which is far superior to that which has been used heretofore. I want to illustrate this point by a personal experience. When I was feebly attempting to enforce the food and drugs act under the restrictions placed upon me, one of the great problems pertained to the oyster industry. At that time the oysters were taken out of salt water several days before they were shipped. They were put in box cars packed with ice, transported, and re-iced when necessary; when they finally reached their destination, half way across the continent or farther, they had absorbed so much fresh water that they ceased to taste like oysters and they were double the size they were when they started. The people who ate them thought what nice fat oysters they were eating. In point of fact, by osmosis the flavor of the oysters had seeped out and fresh water had taken its place. In order to prevent this condition, which was regarded as an adulteration, we issued an order that oysters should not be packed with ice on them, but that the ice be put around the box so that they could be transported without changing their natural flavor.

The shippers of oysters resented this restriction, of course. They did everything they could to have it put aside, but they did not succeed at that time. In order to illustrate to them the desirability of this new way of shipping, I had two samples of oysters taken near Havre de Grace. They were just alike. One of them was packed in the open box

with ice on it, and the other was packed in tight boxes surrounded with ice. Both were the same kind of oysters, and both were shipped to Omaha, re-iced, and then shipped back to Washington. I invited the packers to come down and be the jury. Of course, I knew which were the oysters packed in the tight box and which were the oysters in the open box, and these men themselves ate the oysters and were the jurors. They unanimously agreed that the oysters packed as I have described were superior. They were convinced that it was the only proper way to preserve oysters during transportation. Now, that is one of the practical things which will be improved upon by this dry ice when it is used commercially. It shows that when the chemists prescribe a method of treatment based upon scientific principles which improve the character of the food little by little the makers and transporters of these foods will come to adopt that procedure.

Now, what are these things practically worth? We are in a condition in this country in which agriculture is the complainant. There is a case before the great court of the people of this country, and it is to be decided by the people. I feel that the chemists are in a position to give evidence which will permit a proper decision in the case. What can the chemist do in a little different way toward saving agriculture? I need not stop here to tell you what you already know about agriculture. That has been done by this association now for 43 years, and is still going on. We haven't reached our goal. We haven't reached perfection. The agricultural problem is not solved by any means. You young people who are commencing have just as much to do, just as important work to do as we, your predecessors, had to do. Don't feel that the field is finished. It is only opening. The chemist is capable and willing and powerful enough to do much more valuable work in the future than he has done in the past, and he can help a great deal in solving this agricultural complaint.

I have some statistics that I am about to read, but I think you will pardon me for doing it. I know the value and weakness of statistics. I am well aware of the truth in the witticism that there are lies, damned lies, and statistics. Well, it may be true, but they are handy things to have around once in a while. I have consulted them many times. One statistician states that when the products of the farm come to the consumers of this country they have increased in value about three or four times over what the farmer got for them. He informs us that the farmers of this country get about \$7,500,000,000 for the produce they sell, and when we buy it we pay \$22,500,000,000 for it. That looks like a wide spread of figures—like real statistics by the definition. But I found them correct. For instance, in this part of the country today the

farmer gets about $2\frac{1}{2}$ cents a pound for wheat. We go to the store and buy bread for the table and we pay 11 cents a pound for bread with 40 per cent of water made out of flour with 12 per cent of water. You see that we pay more than four times as much as the farmer gets for it. The same analysis will show that these statistics are not damn lies, but that they are accurate.

Now, the farmer justly complains that he is the only industrialist that is not protected. Now, let us see if that is a fair statement from the farmer's point of view. We have here railways, public utilities, all kinds of transportation facilities, steamboats, busses, and street railways. What do they do? Their directors put a valuation upon the property, and when I look upon it I feel that it is a very generous valuation, indeed. Of course they confirm it. Then the law says that they may earn a fair return on their investment, at least 7 per cent, and so we pay for our transportation on valuations on carriers made by the directors themselves and confirmed by the court and by the law entitling them to a return of 7 or 8 per cent net on their investment. We don't complain about that; it seems a fair thing. Gas, water, and things of that kind are treated the same way, and the telephone and telegraph have the same privilege. Now, right here in this town today the morning paper has an article on the merger of the street railway lines. It is stated that valuations have been placed upon them to the amount of \$63,000,000, but that the companies generously offer to reduce that sum to \$50,000,000, and then to add \$2,000,000 more money immediately. The article goes on to say that at 7 per cent \$3,500,000 should be earned on this valuation, which is a million dollars more than is now earned on a basis of an 8 cent fare. This means that if this merger goes through we shall be assessed a 10 cent or 11 cent fare for the benefit of the owners of public utilities. I ought not to complain about this myself, because I am a stockholder in both these companies. The more they earn, the more I get. Personally, I should like to see it go through, but as a chemist and a philanthropist I do not want to see this go through. The same is true of the Pennsylvania Railroad. They pay 7 per cent now and are authorized by law to do so. In addition to that, they get one-half of the Pullman fare. So you see they get it coming and they get it going. Those are industries that are enabled to live by law in times of high prices.

Now, about the farmers. Every manufacturer places upon his manufactured product the price he can sell it for, and that price includes cost of production, etc. If you want to buy that property you pay that price. They came to my wife yesterday with a proposition to put in a new oil burner for \$500. If we put that particular one in we shall be obliged to

pay that price. I do not know exactly, but it probably costs \$50 to make it. We do not object to all these things—we want everybody to live—but why should everybody object to the farmer making a good living? That is the question the chemist has to answer. As a chemist I have solved the farm problem after thinking about it for years, and I am going to tell you my solution of it, because it is up to the chemists to carry it into effect. They would approve of my plan. I have written it down so as to have it published accurately. First, I shall state the reasons concisely, and then I shall give the bill itself.

EXPLANATION OF THE PROPOSED FARM RELIEF LEGISLATION.

When a manufacturer produces an article, if he is a good business man—and usually he is—he knows exactly what it costs. He puts a price on the article to cover the cost, plus a reasonable profit. There is a kind of free-masonry existing among manufacturers of the same article. The result is that all manufacturers of that particular article fix practically the same price. There is no law authorizing them to do this. There is, however, a principle of ethics which makes it highly advisable that there shall be no price-cutting tactics. Whatever the article may be—a plow, a piece of furniture, an automobile or a threshing machine, a tractor or a teapot—the same principle obtains.

Under present regulations a railroad or a street railway, a bus line or a steamboat line, or any other method of transporting passengers or freight is permitted to submit a statement, with or without the approval of court, showing the value of its plant and the cost of operation. It is then authorized by existing law to charge for this service a fare which will yield the corporation a profit of 6 or 7 per cent. It may well be understood that the value set upon the assets of the company is quite comprehensive and all-embracing. The public, if it desires to ride, pays the fixed fare.

By a similar line of procedure freight charges are set.

Public utilities furnishing gas, electricity, or water are permitted by law to do the same thing and to charge for their services a sufficient sum to earn a generous dividend.

Unionized labor sets the price of both skilled and unskilled labor and the hours of employment. Those who employ labor pay this stipulated price and accept the hours of labor. In addition to this, the importation of laborers from foreign countries is restricted by law. The number of persons learning skilled trades is also limited with the sanction of the law. Unorganized labor profits by the example of organized labor, both as to the amount of compensation and as to the hours of employment. The result is that skilled laborers are often better paid than professors in our colleges and universities, and all this is done by sanction of law.

We come now to the owner or cultivator of agricultural land. By no state or national law, and by no custom, is the owner or renter of agricultural land permitted to set any price on the products he has to sell. He is entirely at the mercy of the broker, the carrier, and the corporation handling, manufacturing, and distributing the products of his labor. The farmer is proverbially adverse to unionization. It is all well enough to say that if the farmer will unionize and cooperate he can eventually fix the price of his own product. If we wait for that event to come about by voluntary action on the part of the farmer, the millennium will beat us to it. Within the limit of one human life no such happy event can be expected. Fortunately, the United States of America

has in every state foundations given by grants of land and money intended for the scientific study of all agricultural problems. The law organizing the Department of Agriculture reads as follows:

"There shall be at the seat of government a Department of Agriculture, the general design and duties of which shall be to acquire and diffuse among the people of the United States useful information on subjects connected with agriculture in the most general and comprehensive sense of that word and to produce, propagate and distribute among the people new and valuable seeds and plants."

With this broad license and mandate Congress has given to the states vast areas of public lands and large sums of money to diffuse in each of the states knowledge of scientific agriculture and agricultural economics in the broadest sense of the word. Agricultural colleges and agricultural experiment stations are now established in every state. They are in full operation. They have as a part of their personnel experts in all the branches of science connected with agriculture, of which chemistry is the chief. They have a body of agricultural economists skilled in the art of determining the cost of agricultural products. These experts are already functioning, and they are capable to the highest degree of reaching sane and scientific conclusions which may guide the farmer in estimating the actual cost of the products of his field. Thus, on broad, scientific, and irrefutable foundations he can proceed to ascertain an approximate cost of the products of his labor. As the farmer is never going to do a thing like this by his own movement and will, it is highly desirable that the authority which first conferred upon him the means of learning scientific agriculture shall proceed to unionize him in a manner by which he can protect his own interests and yet not intrude on the rights of others, by securing a proper profit for his work. By the adoption of the proposed legislation the farmer becomes unionized. He learns the exact cost of his products, and he is in condition to ask a just price for them. He therefore becomes the complete capstone of the arch which already includes all other industries except agriculture. It is high time the farmer should share in the protection given to other industries.

If there is anything in this plan that is repugnant to the Constitution, then all these other methods of unionizing industries are likewise unconstitutional. Why should the Congress of the United States refuse to do for agriculture what it has done for every other industry?

The proposed plan is simple. Any one can understand it. It is also effective. It is not claimed that it will save all farmers from bankruptcy. The protection now afforded the industries does not prevent bad management from resulting in proper punishment. There are failures in banks—even joint land banks—railroad companies, gas works, street railways, bus lines, and occasionally a labor union is disrupted. This plan gives to the farmer the minimum cost of well-directed scientific help. It does not create any additional offices. It utilizes activities that are already established. It does not appropriate any of the taxpayers' money. Any sums used in favoring export are derived from a tariff on like agricultural products. It is the farmers union which ascertains the cost. This plan protects the farmer in securing a fair return. When first adopted it may add somewhat to the cost of living, but eventually it will save the good farmer. It will turn the farm deficit into a profit. It will make agriculture an industry that will attract capital. It will keep capable farmer boys on the farm. It will lead capable farmers' daughters to seek agricultural homes. It recognizes the farmer's wife as worthy of her hire. It will add zest to all efforts of land grant colleges and experi-

ment stations to increase the fertility of the soil. By increasing production the cost of farm products will be lowered, and in the end the great body of non-agricultural consumers, more than 70 per cent of all our people, will get cheaper food. Finally it will make every farmer eager to join the union. His present helpless condition will fade away. As proprietor of the great fundamental industry that is the basis of all other industries, his influence will become dominant.

PROPOSED LEGISLATION FOR FARM RELIEF.

Section 1.—An act to charter a national agricultural union, to stabilize agricultural industries, to enable the farmer to ascertain the cost of the products of his fields, to restrict the importation of competing agricultural products, to increase agricultural efficiency, and for other purposes.

Section 2.—Be it enacted by the House of Representatives and the Senate of the United States in Congress assembled: That agricultural welfare be regarded as affecting the public interest and that it be accorded all the rights and privileges of a public utility; to this end, that an agricultural union shall be chartered in each of the several states, the members of which shall be citizens thereof, and that in favoring this purpose the competent authorities of each state be empowered to request and instruct the trustees of the land grant colleges and the agricultural experiment stations of the various states to select members of the faculties of these colleges and agricultural experiment stations skilled in agricultural economics who shall be the executives of the union. When not less than three of such experts are thus appointed as president, vice-president, and secretary of the union, and not less than twelve *bona-fide farmers* are enrolled as members, said charter is to be given. These officers with such others as may be designated by the union shall determine the cost of production of the following products, namely, milk and milk products, sugar beets, sugar and sorghum cane, sugar and sugar cane and sorghum sirups, all cereals, hay, poultry and eggs, hogs and hog products, beef and beef products, sheep and mutton products, wool, cotton, flax, and any other agricultural staple, not perishable, 75 per cent of which is used within the United States or its insular possessions, which may be added to the above list. These experts shall carefully take into consideration the cost of agricultural products according to established principles of scientific agriculture. They shall not consider the cost of products of so-called "gentlemen farmers", or agricultural estates kept up for show or pleasure. They shall take into consideration the compensation of the farmer himself, of his wife, and of his sons and daughters over fourteen years of age employed on the farm and in the home. Their services shall be valued at the same rate as similar services hired and paid for by the farmer. All hired services shall be included in the cost of production; also all taxes, insurance, and interest on borrowed money; fertilizers, lime, seeds, and inoculating materials; depreciation of implements, fences and buildings; and damage by storm and flood. These experts shall segregate the cost of production so far as possible for each industry mentioned. These officials shall also provide for cooperative buying of farm supplies for the members of the union and for selling farm products, but the members of the union shall pay no fees, nor shall they be assessed in any way for the compensation of the executives. Any additional compensation for these executives shall be provided by the trustees of the land grant colleges and experiment stations with which they are connected.

Section 3.—Having ascertained the cost of production of these staple farm products as nearly as possible for each state, the average cost of all the states shall be determined

with reference also to the quantity produced, so that the mean cost of each individual product for all the states may be known.

Section 4.—The executives of this union are authorized under this charter to ascertain the cost of each of the agricultural products before mentioned and to endeavor to sell collectively at not less than the minimum cost of production, as ascertained above. In addition, to this shall be added the reasonable sum of 10 per cent on the assessed value of land, furniture, harness, live stock, farm implements, and farm buildings, as determined by the assessed value thereof for taxable purposes.

Section 5.—The average cost of producing each article of farm product above mentioned shall be taken as a basis to determine the amount of import duties that shall be levied on all imported articles of the same kind brought into the country for consumption or otherwise, either raw or manufactured, in order to make the cost thereof the same as that of the domestic product. The amount of this tax should be sufficient to equalize the price of the imported article so that it may be upon the same basis of value as the article produced at home. If the subsequent investigations of the cost of production made in the way already provided, and which should be made at least once every two years, should lead to a different cost value, automatically the import duty on the imported article would be changed correspondingly.

Section 6.—When any farmer or tiller of the soil sells the products of his labor direct for exportation to a foreign country, an equalization sum corresponding to the difference between the price paid in the foreign markets and the price ascertained by the union, if the latter should be larger, shall be given to the farmer or tenant selling direct for exportation, provided that this sum shall be taken from the import duties on agricultural products of the same kind fixed as above described; but in no case shall the sum of such equalization items be greater than the total import tax collected on agricultural products of the same kind as the exported article.

Section 7.—This act shall not be construed to prevent the producer of any of the above-mentioned articles, nor any subsequently added thereto, from disposing of said articles at higher prices than those ascertained.

Section 8.—The agricultural union in each state is authorized to arrange for collective selling of farmer's products and collective buying of all farmer's supplies.

That is the whole story. It is a perfectly simple method. Anybody can understand it; anybody can do it. Even McNary and Haugen could understand it, and that is more than they can say of their own bill. I know them both. They are intelligent men. They could see exactly what it means and that it does what we all desire, what I want it to do and what you want it to do. The question is, will they do it? And will your somewhat aged and eternal president get any credit for it? If I could benefit agriculture in this way, I should be perfectly willing to say to our good Lord, "My service is at an end".

SECOND DAY.

TUESDAY—AFTERNOON SESSION.

REPORT ON CEREAL FOODS.

By F. C. BLANCK (Food Research Division, Bureau of Chemistry and Soils, Washington, D. C.), *Referee*.

The past year saw the development of a large amount of additional data from which to reach conclusions regarding the suitability and accuracy of various proposed methods for the analysis of cereal foods. These data, contributed by workers in the regulatory, research, and commercial fields, amply attest to the importance of such studies; they also justify the development and expansion of the program of the association in search of better methods of analysis. It is the belief of the referee that one of the most important functions is to keep all the analytical methods abreast with chemical progress. In order to accomplish this the referees and associate referees must be encouraged to follow the current publication of scientific research and to adapt, modify, and develop procedures applicable to simpler and more accurate cereal analytical methods. The association must depend on the referees and associate referees for guidance in the selection of adequate and suitable methods. The great interest and splendid cooperative spirit manifested by cereal chemists in the development of these methods augurs well for the future.

The referee expresses his grateful appreciation of the exceptional amount of high-caliber work that has been done by the associate referees and collaborators. Without this self-sacrificing effort it would not have been possible to make such rapid progress in clearing up the analytical methods in the cereal field.

SAMPLING OF FLOUR.

The Associate Referee on Sampling of Flour reported a large amount of carefully planned and accurately conducted collaborative work that clearly indicates the accuracy of the tentative method for sampling flour irrespective of whether a single sack or a pile of sacks is sampled. The results also show satisfactory concordance when two individuals sample the same flour.

ASH IN FLOUR.

Associate Referee Coleman reported an exhaustive collaborative study of the glycerol-alcohol modification of the official method which gave results in close agreement with those obtained by the official method.

Further study of the alundum method indicated its undesirability owing to the time required in reaching constant weight. Study of the hydrogen peroxide method brought out various objections to its use. A comparison was also made between the Dutch wet combustion method for ash and the glycerol-alcohol modification of the official method. The Dutch method yielded results averaging 0.072 per cent lower than the official method.

GLUTENIN.

Associate Referee Blish reported on collaborative studies conducted during the year as follows: (1) The Blish-Sandstedt and barium hydroxide methods, and (2) the barium hydroxide method only. In the first of these series, no difference in the probable accuracy of the two methods is indicated. The hydroxide method, however, earns priority by virtue of its simplicity. The second series indicates that some of the differences obtained by the barium hydroxide method may have been due to the fact that the methyl alcohol used was obtained from different sources.

H-ION CONCENTRATION OF FLOUR.

C. H. Bailey, the associate referee on this subject, discussed the collaborative results obtained by Coleman for the A. A. C. C. using the same methods and directions as are employed in the work of this association. In addition, collaborative results were reported on the use of the hydrogen and quinhydrone electrodes. The conclusion is drawn that the difficulties encountered arise in the manipulation of the electrodes and the potentiometer and that there is a greater variation in replicated determinations with the quinhydrone than with the hydrogen electrode.

STARCH IN FLOUR.

Collaborative results reported by Associate Referee Palmer on the proposed modification of the Rask method are promising and worthy of more extended collaborative study. The results by this method should be compared with those obtained by the Hartmann-Hillig modification of the official diastase method.

GLUTEN.

Associate Referee Hertwig secured an appraisal of the value of the method for the determination of gluten to cereal chemists and concluded that work on this method should be discontinued until a more urgent need for its standardization develops.

DIASTATIC STRENGTH OF FLOUR.

Associate Referee Tague reported progress on the studies being made by him on this determination.

CHLORINE IN BLEACHED FLOUR.

Work done on the Seidenberg modification for chlorine in chlorine-bleached flour by Associate Referee Spencer suggested a number of interesting problems. These are being studied and will be reported at the next meeting.

EXPERIMENTAL BAKING TESTS.

Cereal chemists in general regard the baking test as the most important of the laboratory flour tests, particularly in commercially evaluating a flour. A fixed type of procedure with but one variable, the flour, suggests itself as the proper type for a scientific laboratory baking test. Associate Referee Blish, who is also chairman of the Committee on Standardization of the Experimental Baking Test for the American Association of Cereal Chemists, reported distinct progress in the study of the various factors involved in such a method. He believes that a tentative method will shortly be available for collaborative study.

UNSAPONIFIABLE MATTER AND FAT IN FLOUR AND IN ALIMENTARY PASTES.

Samuel Alfend, associate referee, reported extensive collaborative work on these methods, which involved much careful analytical research. As a result of this and previous work directed by this referee, it is believed that no further effort is called for except in the case of the determination of the 40 per cent alcohol precipitable water-soluble protein-nitrogen.

RECOMMENDATIONS¹.

FLOUR.

It is recommended—

- (1) That the tentative method for sampling flour² be adopted as official (first action).
- (2) That the air-oven method³ for the determination of total solids and moisture (indirect method) in flour be adopted as official (final action).
- (3) That the associate referee continue studies on rapid methods for the determination of ash in flour, omitting, however, further consideration of the alundum method.
- (4) That the associate referee study the glycerol-alcohol modification of the official method for the determination of ash in flour with a view to its adoption as a tentative method.
- (5) That the associate referee study the nature and kind of losses occurring when ash is fused.
- (6) That the acid hydrolysis method for the determination of fat in flour⁴ be adopted as an official method (final action).

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1926, 11: 84.

² *This Journal*, 1926, 9: 39.

³ *Ibid.*, 40.

⁴ *Ibid.*, 41.

(7) That the F. A. C. method¹ for the determination of unsaponifiable matter in fats and oils as modified and adopted at the 1926 meeting² be adopted as tentative for flour and subjected to further collaborative study. (This determination is made on the lipoids extracted as directed in the method for the determination of lipoids³.)

(8) That further collaborative studies be conducted involving only the barium hydroxide method for glutenin⁴, with special precautions to insure that all collaborators use the same reagents (which should be of unquestioned purity) and the same procedure in minute detail.

(9) That the tentative method for the determination of hydrogen-ion concentration of flour⁵, amended as recommended by the associate referee by the following addition to the last sentence, "using electrodes and a potentiometric set-up which have been checked through the use of a buffer solution of known hydrogen-ion concentration", be adopted as an official method (first action).

(10) That the associate referee study the possible use of the quinhydrone and antimony electrodes in the determination of the hydrogen-ion concentration of flour.

(11) That further study of the method for the determination of gluten in flour be discontinued until such time as a definite need exists for its standardization and modification.

(12) That the designation "Gluten" for the tentative method on p. 227 of the 1925 edition of *Methods of Analysis* be changed to read "Crude Gluten".

(13) That par. 16, p. 227, of the 1925 edition of *Methods of Analysis*, be amended to read "Quantitative Method.—Tentative (results are approximate)".

(14) That the study of methods for the determination of the diastatic value of flour be continued.

(15) That further study be made on the determination of chlorine in chlorine bleached flour.

(16) That the Rask method for starch⁶, as modified by the associate referee, be adopted as tentative and subjected to further collaborative study.

(17) That the modification of the diastase method for starch suggested by Hartmann and Hillig⁷ be studied.

(18) That the factors for the conversion of the percentages of nitrogen into terms of protein in wheat, wheat bran, wheat endosperm, and wheat embryo suggested by Jones⁸ be adopted.

¹ *This Journal*, 1926, 9: 45.

² *Ibid.*, 1927, 10: 35.

³ *Ibid.*, 1926, 9: 40.

⁴ *Cereal Chem.*, 1927, 4: 129.

⁵ *This Journal*, 1927, 10: 33.

⁶ *Ibid.*, 108.

⁷ *Ibid.*, 1926, 9: 482.

⁸ *Cereal Chem.*, 1926, 3: 104.

BAKED CEREAL PRODUCTS.

It is recommended—

(1) That collaborative study of the tentative method for the preparation of sample of bread¹ be continued.

(2) That the tentative method for the determination of total solids of an entire loaf of bread¹ be further studied.

(3) That the tentative method for the determination of total solids of the air-dried ground sample¹ be further studied.

(4) That studies of the 130°C. air-oven² and other rapid methods for the determination of total solids in an entire loaf of bread be continued.

(5) That comparative studies of the methods for the determination of lipoids (as directed for alimentary pastes³) and of fat in bread be continued.

(6) That the study of methods for the carrying out of experimental baking tests be continued.

(7) That consideration be given to the development of methods for the determination of milk solids in milk bread.

(8) That consideration be given to the development of methods for the determination of rye flour in rye bread.

ALIMENTARY PASTES.

It is recommended—

(1) That the tentative method for taking and preparing a sample of alimentary paste for analysis³ be studied collaboratively.

(2) That the tentative method for the determination of total solids and moisture (indirect method)³ be studied collaboratively.

(3) That the study of the air-oven method for the determination of total solids in flour⁴, as adopted for this determination in alimentary pastes, be continued.

(4) That the tentative acid hydrolysis method for the determination of fat in alimentary pastes, with the slight change suggested by the associate referee, be adopted as official (first action).

(5) That the tentative method for the determination of lipoids and lipid phosphoric acid (P_2O_5) in flour be adopted as official for these determinations in alimentary pastes (first action).

(6) That the F. A. C. method for unsaponifiable matter in fats and oils, as recommended for tentative adoption for flour⁵, be tentatively adopted for the determination of unsaponifiable matter in alimentary pastes.

(7) That the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol⁶ be further studied.

¹ *This Journal*, 1926, 9: 42.

² *Ibid.*, 40.

³ *Ibid.*, 43.

⁴ *Ibid.*, 40.

⁵ *Ibid.*, 45; 1927, 10: 35.

⁶ *Methods of Analysis*, A. O. A. C., 1925, 232.

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REPORT ON SAMPLING OF FLOUR.

By H. RUNKEL¹ (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

The 1926 report² of the associate referee included comments of a number of people interested in a method for sampling flour and proposed experiments to determine the accuracy of the method as applied to one sack of flour as well as to a pile of sacks of flour. The limited data presented indicated that the method was reasonably accurate. Further experimentation was recommended to secure additional data and also to improve the method of sampling a single sack.

This year one modification of the tentative method for drawing the sample from the sack was tried. Instead of drawing one core from each top corner, as provided in the tentative method, one core from the top corner diagonally to the opposite bottom corner of the sack and one from the same top corner, parallel to and one inch from the side to the bottom corner of the sack, were drawn. This work was done by C. B. Stone and A. W. Garrett of the Minneapolis Station, U. S. Food, Drug and Insecticide Administration. A comparison of the moisture content of the samples drawn by the tentative and by the modified method on the same sack is shown as follows:

¹ Presented by F. C. Blanck.

² *This Journal*, 1927, 10: 450.

SACK lbs.		per cent
98	tentative method....	14.06
	modified method....	13.97
	difference.....	0.09
49	tentative method....	14.23
	modified method....	14.30
	difference.....	0.07
140	tentative method....	13.11
	modified method....	13.09
	difference.....	0.02

The difference in results obtained by the two methods was so minor that this proposed modification did not seem to be worth while.

Another modification was tried incidentally by R. C. Sherwood, Director of the Minnesota State Testing Mill, Minneapolis, and A. S. Nordstrum, his assistant. In drawing samples for the experiment to determine how accurately two samplers would check each other, Sherwood took two cores entirely through the sacks from each top corner diagonally to the opposite bottom corner. The results obtained are given in Table 1.

TABLE 1.

Results of Experiment "B" (modified).

(Average of triplicate determinations.)

SACK NO.	SHERWOOD per cent	NORDSTRUM per cent
1	13.60	13.78
2	13.58	13.64
3	14.06	14.03
4	13.84	14.00
5	14.17	14.55
6	13.47	13.65
7	13.45	13.72
8	13.44	13.54
9	13.68	13.45
10	14.30	13.80
Average	13.76	13.82
Difference of averages		0.06

It is noted that the difference in results obtained by the two samplers was only 0.06 per cent. This modification, therefore, was not considered to be a sufficient improvement to justify a change in the tentative method.

As no material improvements were discovered, directions for Experiments "A" and "B", as given in last year's report, were sent out for collaborative work. Six sets of data were received from collaborators representing various organizations, viz., State food and drug officials,

Federal food and drug officials, and agricultural experiment stations. A milling chemist also reported results. The following collaborators took part in the work:

R. O. Baird, L. H. McRoberts, and G. O. Holta, Bismarck, N. D.

F. L. Elliott, Louis L. Judge, Francis X. Colligan, and Bertrand R. Minshall, Baltimore Station.

Joseph Callaway, Jr., Leon A. Salinger, C. F. O'Neill, and L. A. Smith, Savannah Station.

R. L. Horst and J. Y. Breckenridge, New Orleans Station.

The stations mentioned represent the U. S. Department of Agriculture, Food, Drug and Insecticide Administration.

M. J. Blish and R. M. Sandstedt, Agricultural Experiment Station, Lincoln, Nebr.

L. D. Whiting, Louisville, Ky.

In addition to these collaborators, the Cooperative Committee named in last year's report, consisting of M. A. Gray, Leslie R. Olsen, D. A. Coleman, and C. B. Morison, was continued. Many interested collaborators were suggested by the members of this committee.

In addition, Raymond Hertwig of the Hecker H-O Co., Inc., Buffalo, N. Y., studied the method in connection with his own work and advised the associate referee at length concerning his observations. His comments are quite elaborate, but they may be summed up in the one sentence, "I personally would like to see the method adopted as you have drawn it up".

The associate referee wishes to acknowledge the assistance given by all these collaborators, particularly since the work was rather involved and required the time of several men over an appreciable period of time.

DATA TO DETERMINE THE ACCURACY OF THE TENTATIVE METHOD FOR DRAWING A REPRESENTATIVE SAMPLE FROM ONE SACK OF FLOUR.

The directions for collaborative work were given in last year's report, under Experiment "A". One sack of flour was sampled by the tentative method. The flour was mixed in a closed can, resampled by drawing five probes while in the can, and resacked. The sack was immediately resampled by the tentative method, weighed, allowed to stand or lie in a place where loss of moisture might be expected for 5-7 days, and reweighed. It was again sampled by the tentative method, remixed in the closed can and sampled, and then resacked and resampled by the tentative method. All moisture determinations were made in triplicate to eliminate analytical variations. As a further effort to check up on the true moisture content of the sack, the moisture after storage was calculated from the weight change and the moisture on the flour mixed in the closed can before storage. The results obtained are given in Table 2, and those reported last year are included for comparison.

TABLE 2.
Results of Experiment "A".

(To show the accuracy with which the tentative method draws a representative sample from one sack of flour.)

SAMPLER	DATE SAMPLED	MOISTURE (Average of triplicate determinations)				
		(1)	(2)	(3)	(4)	(5)
		Calculated from weight change	Sack sampled by tentative method	Flour mixed in closed can	Resacked flour sampled by tentative method	Difference— "Sack sampled by tentative method" (Col. 2) and "Flour mixed in closed can" (Col. 3)
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
C. F. O'Neill ^a	9-28-27	...	12.84*	12.86*	12.95*	0.02
	10- 4-27	13.29	12.98	13.15	13.13	0.17
L. H. McRoberts ^c	4-11-27	...	14.75	14.69	14.72	0.06
	4-18-27	12.66 ^d	12.31	12.91 ^d	12.87	0.60 ^e
B. R. Minshall ^f	3- 5-27	...	14.19	14.02	14.05	0.17
	3-12-27	12.55	12.51	0.04 ^g
R. L. Horst	7-18-27	...	13.87	13.99	13.93	0.12
	7- 7-27	13.82	13.89	13.94	13.92	0.05
R. M. Sandstedt ^h	7-19-27	...	13.00	13.04	12.92	0.04
	7-27-27	12.53	12.53	12.27	12.24	0.26
L. D. Whiting ^g	Stored
	5 days	...	13.28	13.38	13.38	0.10
H. Runkel ^m	9- 3-26	...	13.70	13.78	13.65	0.08
	9-28-26	12.82	12.83	12.81	12.81	0.02
	10- 8-26	11.88	12.26	12.16	11.81	0.10
F. A. Collatz ⁿ	9-24-26	13.46	13.48	...
	10- 4-26	12.22	12.60	12.15	12.22	0.45
					Average	0.12

* Average of duplicates.

^a Analysis by Joseph Callaway, Jr.

^c Holta assisted.

^d Rise in moisture explained by fact that sack had to be carried from laboratory (humidity 40%) to warehouse where humidity was 55-60%.

^e Weights probably increased because of transfer to warehouse (See *d*).

^f Analysis by F. L. Elliott.

^h Results submitted by M. J. Blish. Single moisture determinations reported.

^g Included from last year's report for comparison.

ⁿ Compared with moisture calculated from weight change.

^o Not comparable because of change in moisture between time of first sampling and mixing in can.

^m Analysis by Spencer method at 130°C. for 1 hour.

In the seven entries in Table 2 of the moisture content, "Calculated from weight change" (Column 1), the differences from the moisture on the "Flour mixed in closed can" (Column 3) are 0.14, 0.25, 0.12, 0.26, 0.01, 0.28, and 0.07 per cent. These differences may be partly accounted for by the inaccuracy of scales since a variation of 1 ounce would make a difference of about 0.20 per cent on a 50-pound sack.

It may also be noted that the differences between the moisture results on "Resacked flour sampled by tentative method", and the results on "Flour mixed in closed can" (see Columns 4 and 3), in order from top of table are: 0.09, 0.02, 0.03, 0.04, 0.03, 0.06, 0.02, 0.12, 0.03, 0.00, 0.13, 0.00, 0.35, 0.02, and 0.07 per cent. With the exception of 0.35 per cent, where the sack was stored for more than 1 month, the slight variations tend to confirm the belief that the results on the sample "Mixed in closed can" are very near the true moisture content of the sacks at the time of sampling.

The differences between results on "Flour mixed in closed can" (Column 3) and results obtained by "Sack sampled by tentative method" (Column 2) are shown in Column 5.

From Column 5 it is noted that the widest variation between the probable true moisture content and the moisture content found by the tentative method is 0.45 per cent, except in one instance in which the collaborator indicates that the transfer of the flour from the laboratory to the warehouse for sampling raised the moisture content. The next highest difference is 0.26 per cent, and the other variations are reasonably small. The average variation is 0.12 per cent.

In order to make comparisons between analytical and sampling variations, 71 sets of triplicate determinations made on the same sample and reported in this work were compared. The widest variation found in any of these 71 sets was 0.28 per cent, the next widest was 0.21 per cent, and the average was 0.085 per cent. About two-thirds of the variations were less than 0.10 per cent. Comparing these differences in the analytical and sampling results, as reported in Column 5, it is noted that the tentative method gave average variations from the probable true moisture content that are only approximately one and one-half times the size of the average analytical variations and that in the majority of instances the sampling variations and analytical variations are practically the same. Accordingly, the tentative method is believed to be reasonably accurate for sampling a single sack of flour.

**DATA TO SHOW THE ACCURACY WITH WHICH ONE SAMPLER MAY
CHECK ANOTHER BY USING THE TENTATIVE METHOD
ON THE SAME PILE OF FLOUR.**

Directions for the collaborative work were given in last year's report under Experiment "B". Two men simultaneously sampled the same pile of flour by the method, making the analyses in triplicate. The results are given in Table 3. Sacks Nos. 1-4 were from the most exposed portion of the pile, Nos. 5-7 from the next less exposed portion, Nos. 8 and 9 from the next less exposed portion, and Sack No. 10 from the least exposed portion of the pile. Different sacks from each pile of flour were selected by the two samplers. The averages of the experiments reported last year are included for comparison.

TABLE 3.

Results of Experiment "B".

(To show the accuracy with which one sampler may check another on the same pile of flour by the use of the tentative method.)

MOISTURE						
(Average of triplicate determinations.)						
LOCATION OF PILE	SACK NO.	O'NEILL per cent	SMITH per cent	LOCATION OF PILE	JUDGE per cent	COLLIGAN per cent
Savannah, Ga. ^a	1	13.15	13.33	Baltimore, Md. ^d ^e	13.63	14.05
	2	12.83	13.29		13.78	13.04
	3	13.04	13.31		13.82	13.77
	4	12.87	13.42		13.70	13.82
	5	12.99	13.03		13.63	13.62
	6	12.94	13.00		13.69	13.54
	7	12.93	13.21		13.68	13.55
	8	12.86	12.89		13.81	14.02
	9	13.00	13.10		13.76	13.99
	10	12.90	12.81		13.85	13.84
Average	12.95	13.14		13.72		
Difference		0.19			0.02	
		MCROBERTS	HOLTA		SANDSTEDT "1" ^a	SANDSTEDT "2" ^a
Russell Miller Milling Co., (Warehouse), Mandan, N. D. ^b	1	14.23	14.31	Lincoln, Nebr.	12.98	13.07
	2	13.94	14.27		13.06	13.06
	3	14.48	14.34		13.18	13.03
	4	14.30	14.38		13.50	13.16
	5	14.31	14.63		13.10	13.11
	6	14.30	14.27		13.06	13.06
	7	14.70	14.50		13.15	13.35
	8	14.30	14.18		13.39	13.14
	9	14.26	14.37		13.30	13.37
	10	14.10	14.24		13.47	13.40
Average	14.29	14.35		13.22	13.18	
Difference		0.06			0.04	
		HORST	BRECKEN- RIDGE		WHITING "1" ^a	WHITING "2" ^a
Pillsbury Flour Mills, (Warehouse), New Orleans, La. ^c ^e	1	13.23	13.55	Ballard & Ballard Mills, Louisville, Ky. ^e	13.15	13.21
	2	13.82	13.69		13.10	13.11
	3	13.67	13.67		13.34	13.13
	4	13.60	13.50		12.98	13.20
	5	13.25	13.70		13.30	13.14
	6	13.56	13.18		12.93	13.09
	7	13.01	13.73		12.90	13.06
	8	13.34	13.69		13.16	12.97
	9	13.73	13.89		13.15	13.15
	10	13.90	13.67		13.06	13.17
Average	13.51	13.63		13.11	13.12	
Difference		0.12			0.01	
		COLLATZ "A" ^a	COLLATZ "B" ^a			
Washburn Crosby Mill ^f , Minneap- olis, Minn.	1-10					
	Average	13.45	13.46			
	Difference		0.01			
		GRAY "1" ^a	GRAY "2" ^a			
Pillsbury Mill ^f , Minneapolis, Minn.	1-10					
	Average	13.38	13.40			
	Difference		0.02			
		GARRETT	GILL			
Duluth Universal Mill ^f , Duluth, Minn.	1-10					
	Average	13.66	13.61			
	Difference		0.05			

^a Analyses by L. A. Salinger.^b Analyses by L. H. McRoberts.^c Analyses by R. L. Horst.^d Analyses by F. L. Elliott.^e Single moisture determinations.^f From last year's report for comparison.^g Order of sacks as to exposure not actually reported but believed to be in order directed.

The differences between the duplicate samples in the nine sets in Table 3 are 0.19, 0.06, 0.12, 0.02, 0.04, 0.01, 0.01, 0.02, and 0.05 per cent. The average difference is 0.06 per cent. These results indicate that the method gives reasonably close checks on two samples from the same pile of flour when used by two different samplers.

SUMMARY.

The method has been considered favorably by various trade and official organizations (see previous reports 1925¹–1926²). The collaborative data submitted cover variations in results when the method is applied to one sack of flour and when applied to a pile of sacks of flour. Reasonably small variations are reported in each case. The method was adopted as tentative last year. It is, therefore, recommended that the method be adopted as official³.

REPORT ON ASH IN FLOUR AND GASOLINE COLOR VALUE.

By D. A. COLEMAN (Bureau of Agricultural Economics, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*.

The activities of the Associate Referee on Ash in Flour and Gasoline Color Value were similar to those carried on last year. Special attention was given to the so-called "short methods" for ashing flour, as well as to comments reaching the referee concerning the technic of the various methods reported on previously.

Last year's investigations showed that the temperature 550°C., recommended by this association for ashing flour, was satisfactory providing the flour did not ash down to a hard carbonaceous mass. It was pointed out that this concentration of carbon particles could be prevented by adding and mixing with the flour a small quantity of glycerol-alcohol or fine (40-mesh) alundum.

However, subsequent trials with alundum in the hands of other people did not give satisfaction. The ash results obtained sometimes agreed with the A. O. A. C. method, but on many occasions there was a complete lack of agreement. Investigation was made into the cause of this, and it was found that ignited alundum returned to a constant weight so slowly after heating that a shifting base was continually present, making accurate results impossible until complete equilibrium had resulted. The speed of reaction was found to be in direct proportion to the quantity of alundum used. With a 1 gram charge of alundum, approximately 1 hour was consumed in reaching constant weight. With a 2 gram charge, from 1 to 1½ hours was necessary. Due to this fact, therefore, it was

¹ *This Journal*, 1926, 9: 423.

² *Ibid.*, 1927, 10: 450.

³ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 84.

decided to eliminate the use of this material from further study as a means to the rapid and satisfactory ashing of flour.

The only criticism regarding the use of glycerol-alcohol expressed by various chemists was the time consumed and the attendant errors when this mixture was mixed with the flour.

Earlier tests in the referee's laboratory showed that this thorough mixing of glycerol-alcohol was unnecessary, it being sufficient to place the glycerol-alcohol mixture on top of the flour in the ashing crucible, complete wetting of the flour mass being accomplished in less than 10 minutes. The subsequent ignition of the alcohol and heating in the muffle generates enough vapor to thoroughly permeate the flour mass. With time and error eliminated collaborative data were sought regarding the comparative efficiency of the two methods, i. e., the official A. O. A. C. at 550°C. and the same method modified to allow the use of glycerol-alcohol (50-50 solution) in the amount of 1.5 cc. of glycerol-alcohol to each gram of flour.

Reports on the ash content of 5 samples of flour given by 52 collaborators are shown in Table 1. The results obtained in this study showed that the glycerol-alcohol modification can be carried out with the same degree of accuracy and efficiency as can the A. O. A. C. method without addition of glycerol-alcohol.

The average ash content obtained by the 52 collaborators on the five samples of flour was 0.475, 0.687, 0.551, 0.579, and 0.553 per cent. With the use of glycerol-alcohol the percentage was 0.478, 0.687, 0.555, 0.583, and 0.557 per cent. It would appear, therefore, inasmuch as the accuracy of the A. O. A. C. method is not sacrificed by the addition of glycerol-alcohol and that it is a known fact that glycerol-alcohol will prevent the ash from forming into a hard carbonaceous mass, that its use should be allowed, at least when difficultly ashable flours are encountered.

Since last year's meeting, Sullivan and Near¹ have suggested the use of 5 per cent hydrogen peroxide as an aid to the rapid ashing of flour. These investigators claim that by the use of this material they obtain "in a shorter time a cleaner, whiter ash on all flours, wheat, or bran". The time of heating ranged from 12 to 16 hours. Briefly, their method, directions for which are by no means specific, calls for thoroughly moistening the sample to be ashed with 5 per cent hydrogen peroxide, drying the material at a low temperature so that the material becomes fairly dry before raising the muffle to the ashing temperature, 610-620°C., and igniting for 12-16 hours. Although the time element immediately eliminates this method from the rapid method class, it was thought worth while to run some ash tests with it to see how it compared with the A. O. A. C. method and the suggested glycerol-alcohol modification of the A. O. A. C. method.

¹ *J. Am. Chem. Soc.*, 1927, 49: 467.

TABLE 1.

Ash content of samples of wheat flour sent out for collaborative study.

COLLABORATOR	ASSED BY A. O. A. C. METHOD					ASSED BY MODIFIED* A. O. A. C. METHOD				
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.483	0.685	0.556	0.590	0.559	0.484	0.686	0.559	0.591	0.559
2	0.473	0.696	0.556	0.584	0.555	0.480	0.695	0.556	0.589	0.557
3	0.447	0.687	0.527	0.578	0.550	0.463	0.667	0.510	0.587	0.540
4	0.470	0.680	0.540	0.580	0.550	0.460	0.660	0.530	0.580	0.550
5	0.463	0.666	0.566	0.573	0.558	0.523†	0.711†	0.596†	0.593†	0.588†
6	0.487	0.692	0.555	0.585	0.553	0.477	0.691	0.555	0.585	0.555
7	0.483	0.693	0.560	0.587	0.560	0.480	0.693	0.560	0.587	0.560
8	0.465	0.676	0.543	0.580	0.546	0.477	0.681	0.545	0.575	0.556
9	0.473	0.697	0.555	0.583	0.560	0.473	0.700	0.560	0.590	0.583
10	0.474	0.691	0.559	0.573	0.544	0.488	0.701	0.562	0.592	0.566
11	0.480	0.692	0.552	0.585	0.556	0.482	0.693	0.555	0.588	0.557
12	0.473	0.679	0.553	0.579	0.556	0.481	0.684	0.557	0.585	0.563
13						0.462	0.682		0.570	0.555
14	0.488	0.695	0.555	0.585	0.568	0.495	0.692	0.556	0.592	0.555
15	0.480	0.695	0.560	0.585	0.560	0.480	0.690	0.560	0.580	0.555
16	0.460	0.677	0.534	0.578	0.548	0.476	0.694	0.555	0.582	0.561
17	0.468	0.676	0.546	0.584	0.546	0.482	0.774†	0.570	0.588	0.566
18	0.460	0.671	0.476†	0.516†	0.486†	0.466	0.650	0.555	0.507†	0.526
19	0.483	0.698	0.563	0.573	0.548	0.465	0.688	0.568	0.585	0.548
20	0.470	0.685	0.558	0.582	0.555	0.475	0.673	0.557	0.585	0.558
21	0.481	0.683	0.551	0.588	0.561	0.484	0.698	0.555	0.603	0.558
22	0.475	0.688	0.552	0.583	0.554	0.472	0.690	0.552	0.585	0.559
23	0.484	0.692	0.554	0.587	0.553	0.466	0.679	0.555	0.570	0.545
24	0.459	0.668	0.527	0.556	0.538	0.458	0.645	0.541	0.571	0.549
25	0.473	0.686	0.556	0.580	0.563	0.480	0.690	0.565	0.596	0.560
26	0.485	0.696	0.551	0.573	0.546	0.503	0.705	0.554	0.583	0.570
27	0.464	0.670	0.544	0.568	0.544	0.476	0.688	0.564	0.582	0.568
28	0.461†	0.657†	0.483†	0.557†	0.517†	0.465	0.657	0.576	0.553	0.530
29	0.473	0.689	0.548	0.577	0.549	0.472	0.688	0.547	0.581	0.551
30	0.475	0.697	0.555	0.565	0.555	0.475	0.690	0.550	0.560	0.550
31	0.470	0.673	0.545	0.587	0.560	0.476	0.683	0.553	0.590	0.570
32	0.455	0.666	0.563	0.573	0.548	0.485	0.668	0.568	0.585	0.548
33	0.480	0.690	0.565	0.590	0.575	0.475	0.695	0.575	0.600	0.580
34	0.478	0.688	0.544	0.580	0.546					
35	0.482	0.687	0.548	0.580	0.552	0.485	0.693	0.560	0.583	0.557
36	0.477	0.685	0.541	0.581	0.545	0.470	0.683	0.558	0.570	0.548
37	0.470	0.688	0.534	0.584	0.554	0.476	0.688	0.540	0.584	0.560
38	0.480	0.697	0.563	0.589	0.567	0.484	0.700	0.573	0.600	0.573
39	0.470	0.676	0.539	0.580	0.549	0.474	0.674	0.540	0.550	0.533
40	0.471	0.679	0.558	0.494	0.556	0.470	0.686	0.555	0.481†	0.556
41	0.477	0.684	0.553	0.575	0.553	0.480	0.696	0.561	0.591	0.564
42	0.470	0.687	0.557	0.583	0.553	0.483	0.698	0.577	0.597	0.573
43	0.477	0.690	0.557	0.563	0.545	0.485	0.680	0.540	0.570	0.553
44	0.479	0.693	0.559	0.581	0.550	0.475	0.694	0.558	0.582	0.556
45	0.500	0.700	0.550	0.580	0.550	0.495	0.695	0.545	0.575	0.545
46	0.480	0.693	0.550	0.575	0.563	0.485	0.685	0.560	0.585	0.560
47						0.476	0.686	0.550	0.580	0.550
48	0.487	0.715	0.546	0.565	0.542	0.486	0.704	0.552	0.568	0.551
49	0.480	0.697	0.557	0.580	0.557	0.457	0.680	0.553	0.577	0.540
50	0.494	0.695	0.564	0.562	0.547	0.490	0.697	0.580	0.590	0.570
51	0.474	0.690	0.540	0.570	0.550	0.483	0.683	0.533	0.567	0.550
52	0.475	0.685	0.545	0.579	0.551	0.484	0.696	0.566	0.590	0.564
53	0.473	0.693	0.556	0.593	0.563	0.470	0.693	0.560	0.590	0.570
54	0.476	0.680	0.570	0.576	0.553	0.480	0.686	0.566	0.583	0.556
55	0.490	0.710	0.533	0.570	0.533					
56†	0.530	0.700	0.605	0.630	0.600	0.500	0.700	0.537	0.580	0.540
Samples	52	52	51	51	51	52	51	51	50	52
Average	0.475	0.687	0.551	0.579	0.553	0.478	0.687	0.555	0.583	0.557
Maximum	0.500	0.715	0.570	0.593	0.575	0.503	0.704	0.577	0.603	0.580
Minimum	0.447	0.666	0.527	0.562	0.533	0.462	0.645	0.510	0.560	0.526
Range	0.053	0.049	0.043	0.031	0.042	0.041	0.059	0.067	0.043	0.054

* Glycerol-alcohol.

† Omitted from averages.

Accordingly, twelve spring wheat, straight-grade flours known to be difficult to ash by the A. O. A. C. method were selected and ashed: (1) by the A. O. A. C. method; (2) by the glycerol-alcohol modification of the A. O. A. C. method; (3) by the peroxide method, ashing at a temperature of 550°C.; and (4) by the peroxide method, ashing at 615°C. The results obtained are given in Table 2.

TABLE 2.

Comparison of the A. O. A. C., the modified A. O. A. C., and the hydrogen peroxide methods for ashing flour.

SAMPLE NO.	A. O. A. C. METHOD	A. O. A. C. METHOD* MODIFIED	H ₂ O ₂ at 550°C.	H ₂ O ₂ at 615°C.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
12	0 666	0.653	0 659	0.657
22	0.591	0.597	0 584	0 591
23	0 526	0 526	0 521	0 531
24	0 613	0 616	0 607	0 615
25	0 500	0 468	0 460	0 474
26	0 544	0 530	0 530	0 528
27	0 603	0 593	0 603	0 601
28	0 560	0.570	0 555	0.562
29	0 577	0 568	0 603	0 594
31	0 557	0 550	0 568	0 567
34	0 481	0 474	0 488	0 491
50	0 555	0 560	0 518	0 543
General Average	0 564	0 558	0 558	0 563

* Glycerol-alcohol.

The results obtained on these twelve flours by all four methods were practically the same. The average ash content of the twelve flours by each of the four methods was 0.579, 0.573, 0.572, and 0.578 per cent, respectively.

However, there were marked differences in the appearance of the ash. The ash of those samples ashed by the A. O. A. C. method without the use of glycerol-alcohol was not satisfactory in appearance; nine were very dark gray and three were black in color. At 550°C. the peroxide method gave an ash considerably better in appearance than that obtained by the A. O. A. C. method without additions, but on the average somewhat darker in color than the ash obtained by the addition of glycerol-alcohol.

When a higher temperature was used with the peroxide method, namely, 615°C., all ash was fused.

The peroxide method has all the criticisable features of the original glycerol-alcohol procedure; in fact, it has more. The peroxide is more difficult to mix with the flour, and the mixing consumes more time. It does not wet as easy as glycerol-alcohol. The flour has to be dried to a large extent before ignition. The recommended temperature of ignition is too high, because the ash fuses at this temperature. For these various reasons, this method was eliminated from collaborative study.

As a final activity the associate referee made a few comparative tests to determine the variation in results between the Dutch Government

method for ashing flour and the A. O. A. C. method. This work was started because complaints were received by the trade regarding the ash content of American export flours as tested in Dutch Government laboratories.

Eleven of the flours reported in Table 2 were ashed according to the Dutch method, which is as follows:

Grind 5 grams of flour to powder. Mix with some water. Add 1 cc. of 3 *M* sulfuric acid. Remove surplus of acid by heating mixture moderately. Cool mixture and add a few drops of sulfuric acid. Heat again. During calorification add some pieces of ammonium carbonate. Weigh residue cold. To determine ash, multiply sulfate ash by 8/9.

The results obtained by the Dutch method are shown in Table 3.

TABLE 3.

Comparison of the modified A. O. A. C. method and the Dutch Government method for ashing flour.

SAMPLE NO.	GLYCEROL-ALCOHOL METHOD	DUTCH GOVERNMENT METHOD	DIFFERENCE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
12	0.653	0.582	-0.071
22	0.597	0.518	-0.079
23	0.526	0.450	-0.076
24	0.616	0.526	-0.090
26	0.530	0.457	-0.073
27	0.593	0.544	-0.049
28	0.570	0.481	-0.089
29	0.568	0.497	-0.071
31	0.550	0.481	-0.069
34	0.474	0.416	-0.042
50	0.560	0.478	-0.082
General Averages	0.567	0.493	-0.072

The average difference by the two methods is 0.072 of 1 per cent, individual differences depending upon the flour ranging from -0.042 of 1 per cent to -0.090 of 1 per cent. The A. O. A. C. method modified by the addition of glycerol-alcohol gives higher results in every instance.

Owing to lack of time no studies were made regarding the methods for making gasoline color value determinations on flour. Likewise, no investigations were made as suggested by the referee last year concerning the nature and kind of losses occurring when flour is ashed.

SUMMARY AND RECOMMENDATIONS¹.

The use of alundum should be eliminated as a factor in the rapid ashing of flour owing to the difficulty in obtaining constant weight after ignition.

The addition of glycerol-alcohol to flour for the purpose of hastening the ashing process and for the prevention of the formation of hard car-

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 84.

bonaceous masses with certain classes and grades of flour should be permitted.

The use of 5 per cent hydrogen peroxide as an aid to the rapid and satisfactory ashing of flour has no advantage over glycerol-alcohol. The temperature to be used with this method is too high as it causes the ash to fuse.

An average difference of 0.072 of 1 per cent was found in the ash content of straight-grade flour when ashed by the A. O. A. C. method and the Dutch Government method.

REPORT ON GLUTENIN IN FLOUR.

By M. J. BLISH (Agricultural Experiment Station, Lincoln, Nebr.),
Associate Referee.

Following the recommendations based upon last year's report¹, the work of the past season was confined to collaborative studies of two methods which had been found in this laboratory to yield results agreeing as closely as may reasonably be expected in present-day methods of protein fractionation. These are the Blish-Sandstedt method², and the so-called barium hydroxide method³. These two methods, in turn, yield figures which agree fairly well with values obtained by the Sharp and Gortner⁴ procedure, the latter being based upon the generally accepted principles established by Osborne⁵. Of these procedures, the barium hydroxide method is the simplest, while that of Sharp and Gortner is the most complicated.

COLLABORATIVE STUDIES.

During the past year two series of collaborative studies were undertaken. The first of these series was conducted by D. A. Coleman⁶, chairman of the Committee on Methods of the American Association of Cereal Chemists. Two samples of flour, of high and low protein content, respectively, were submitted to each of 10 collaborators. Each collaborator estimated glutenin in these samples by both the Blish-Sandstedt and the barium hydroxide methods. In addition, three collaborators made determinations by the Sharp and Gortner method. In sending out the samples Coleman furnished to each collaborator the necessary reagents for use in the barium hydroxide method. The results of this collaborative study are shown in Table 1.

Inspection of Table 1 reveals that the results are by no means as concordant as might be desired. This is partially due, however, to a sur-

¹ *This Journal*, 1927, 10: 465.

² *Cereal Chem.*, 1925, 2: 57.

³ *Ibid.*, 1927, 4: 129.

⁴ *Minnesota Tech. Bull.* 10, 1923.

⁵ *The Proteins of the Wheat Kernel*. Carnegie Institution of Washington, 1907.

⁶ *Cereal Chem.*, 1927, 4: 311.

TABLE 1.

Results of a collaborative study of methods for estimating glutenin in flour conducted by Coleman.

COLLABORATOR	TOTAL PROTEIN		GLUTENIN (BLISH-SANDSTEDT METHOD)		GLUTENIN (BARIUM- HYDROXIDE METHOD)		GLUTENIN (SHARP- GORTNER METHOD)	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	14.08	8.20	4.50	2.58	5.00	2.57
2	14.51	8.12	5.58	3.19	5.49	3.51	5.00	3.13
3	14.23	7.95	5.13	2.83	5.45	3.28	5.15	3.28
4	14.27	7.94	5.15	2.91	5.35	3.27		
5	14.46	8.05	4.84	2.73	5.55	3.57		
6	14.34	7.98	5.12	2.93	5.21	3.22	5.26	3.05
7	14.93	8.21	5.26	2.88	5.58	3.31		
8	14.64	8.27	4.94	2.72	5.98	3.62		
9	14.68	8.08	5.38	3.35	5.41	3.32		
10	14.36	8.10	5.18	3.32		
Average	14.45	8.07	5.10	2.94	5.41	3.30	5.14	3.15
Maximum	14.93	8.27	5.58	3.35	5.98	3.57	5.26	3.28
Minimum	14.08	7.94	4.50	2.58	5.00	2.57	5.00	3.05
Range	0.85	0.33	1.08	0.77	0.98	1.00	0.26	0.23

prising variation in the figures for total protein in the flour, especially in the one of high protein content. Taking this fact into account and eliminating the two extremes in the averages, it will be noted that the concordance of results is, for the most part, as good as may be expected in a method that involves protein separations. Averages of results by both methods agree fairly well with the averages of the results obtained by the Sharp and Gortner method. In the case of the high protein flour, the results by the Blish and Sandstedt procedure agree more closely with those obtained by the Sharp and Gortner method than do those secured by the barium hydroxide method. The reverse is true in the case of the low protein flour. In both flours the results secured by the barium hydroxide method average slightly higher than those obtained by the Blish and Sandstedt method.

This study does not indicate a preference for either method from the standpoint of probable accuracy. Both appear to be reasonably accurate with the majority of the collaborators. The barium hydroxide method deserves preference if simplicity of operation is considered.

A second collaborative study, conducted by the associate referee, involved the barium hydroxide method alone, as used by 11 collaborators with one sample of flour of approximately average protein content. Each collaborator was supplied with a sample of flour and of barium hydroxide from the same lots, respectively, although methyl alcohol from the same source was not used by all collaborators. The results of this series of tests are shown in Table 2.

TABLE 2.

Results of a collaborative study of the barium hydroxide method for estimating glutenin conducted by the associate referee.

COLLABORATOR	TOTAL PROTEIN	GLUTENIN
	<i>per cent</i>	<i>per cent</i>
1	11.35	4.27
2	11.40	4.19
3	11.40	4.37
4	11.84	4.85
5	11.34	5.03
6	11.34	4.59
7	11.44	4.11
8	11.38	4.04
9	11.34	3.99
10	11.34	3.79
11	11.34	4.19
Average	11.41	4.31
Maximum	11.84	5.03
Minimum	11.34	3.79
Range	0.50	1.24

In the series shown in Table 2, 7 of the 11 collaborators obtained results which the writer would regard as acceptably concordant. One result is obviously too low, while three are too high. One of the high results is apparently due largely to the high value which that particular collaborator obtained for the total protein in the flour, upon which the final calculation is based. In all other instances the figures for total protein check very closely.

Some of the variations in results may have been due to the use of methyl alcohol obtained from different sources. It is quite conceivable that an appreciable quantity of ketones or aldehydes, if present in methyl alcohol as is sometimes the case, might react with certain chemical groups in proteins in such a manner as to alter their solubilities. This possibility should be investigated and taken into account in further work with methods involving the use of methyl alcohol.

INDIVIDUALITY OF GLUTENIN.

Attention is again called to the fact that the work thus far has necessarily been based upon the general acceptance of the validity of Osborne's characterization of wheat glutenin. From time to time, however, doubt has arisen as to the chemical identity and individuality of this protein. Quite recently Csonka and Jones¹ have reported evidence indicating that two glutenin fractions of different chemical individualities may be isolated from wheat flour by fractional precipitation with ammonium sulfate. These are designated, respectively, as α -glutelin and β -glutelin. Both glutelins apparently have the same isoelectric point, as well as the same general solubility characteristics, and it may reasonably be assumed

¹ *J. Biol. Chem.*, 1927, 73: 321.

that they are estimated together by any of the three methods discussed in this, as well as in previous reports.

In the light of the recent findings of Csonka and Jones, and until further information is available, it now appears that glutenin may be regarded as a mixture of two proteins having similar properties. Since present quantitative methods for glutenin probably account for both the alpha and beta forms, the question naturally arises as to whether or not the ratio of one variety to the other is the same in all flours. This point will require further investigation.

RECOMMENDATIONS¹.

It is recommended that further collaborative studies be made, involving only the barium hydroxide method for glutenin, with special precautions to insure that all collaborators use the same reagents (which should be of unquestioned purity) and the same procedure in minute detail.

REPORT ON HYDROGEN-ION CONCENTRATION OF FLOUR.

By C. H. BAILEY (Agricultural Experiment Station, St. Paul, Minn.),
Associate Referee.

Last year the associate referee cooperated with the Committee on Methods of the American Association of Cereal Chemists in organizing a program of the study of the determination of the hydrogen-ion concentration of flour. In consequence, the same directions for work were sent to the collaborators of both the A. O. A. C. and the A. A. C. C. The instructions were not changed materially this year, and therefore the collaborators again used identical procedures.

The results of the study conducted by D. A. Coleman for the American Association of Cereal Chemists have been published². Coleman distributed a phthalate buffer solution and three samples of flour. After computation, the coefficient of variation of the results reported for the buffer solution and Coleman's flour sample No. 1 were found to be 15.08 per cent and 2.69 per cent, respectively; thus the relative variability in the instance of the reports on the buffer solutions was substantially greater than in the case of the flour sample. Reference will be made later in this report to the bearing of this fact upon the development of a method for flour.

In 1926 the associate referee distributed a sample of straight-grade flour for the A. O. A. C. collaborative studies.

The following instructions accompanied these samples:

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 85.

² *Cereal Chem.*, 1927, 4: 321.

The method to be employed in the electrometric determination of hydrogen-ion concentration is as follows:

Weigh 10 grams of the flour into a 250 cc. flask and add 100 cc. of neutral distilled water at 25°C. Immediately suspend the flour particles by whirling or agitating the contents of the flask. Maintain at 25°C. for 30 minutes, shaking at 5 minute intervals in order to keep the flour particles in suspension. Allow to stand exactly 10 minutes to allow the flour particles to settle, decant the supernatant liquid into the electrode vessel, and at once determine the hydrogen-ion concentration electrometrically.

The referee urges that the potentiometer set-up be checked through the use of an appropriate buffer solution before the flour extract is tested.

It is anticipated that the collaborators are provided with hydrogen electrode vessels and will ordinarily complete the determination through the use of such an electrode. The associate referee desires that in addition to such determinations the collaborators, if possible, also determine the hydrogen-ion concentration of an aliquot of the same extract through the use of a quinhydrone electrode. In the event that quinhydrone is not available to the collaborators, the referee on being advised will forward the necessary quantity of this reagent.

The set-up to be used in such a determination can be made very simply to consist of a small glass vial into which a small strip of bright platinum is placed, which platinum is connected to the positive (+) pole of the E. M. F. binding posts of the potentiometer. A siphon tube may be used to connect this vessel to the salt bridge. Five to ten cc. of the flour extract (depending upon the size of the vial) is sufficient and this must be saturated with quinhydrone by introducing an excess of the dry powder and stirring or agitating intermittently over a period of 3-4 minutes. If an excess of quinhydrone is present, it will settle out and form a dark layer in the bottom of the vessel. A saturated calomel electrode should be used as the other half-cell, and is connected to the minus (-) pole of the E. M. F. binding posts of the potentiometer. If a more elaborate set-up is desired, the arrangement of vessels used by Denham and Blair¹ may be employed. The hydrogen-ion concentration as pH at 25°C. with this arrangement of half-cells will equal

$$\text{pH} = \frac{454.5 - \text{E. M. F.}}{59.16}$$

In reporting results please indicate the characteristics of the calomel electrode and hydrogen electrode vessels used as well as a diagram of the set-up used with the quinhydrone electrode. Also record the temperature observed in the room at the time both sets of measurements are made.

Eight collaborators reported results obtained with the hydrogen electrode and four of these reported results obtained with the quinhydrone electrode. These data appear in Table 1. A statistical analysis of the data obtained through the use of the hydrogen electrode shows that the coefficient of variation is 1.63 per cent. This is about one-third the coefficient of variation, 5.00 per cent, given last year in the report of twelve collaborators on patent flour². Seemingly an improvement in the technic of the operators is evident from these findings.

Last year the associate referee distributed a buffer solution to the several collaborators, and there was a substantial variation in the hydro-

¹ *Cereal Chem.*, 1926, 3: 159.

² *This Journal*, 1927, 10: 469.

TABLE 1.

Results of the determination of the hydrogen-ion concentration (as pH) of a straight grade flour sample distributed by the associate referee.

COLLABORATOR NO.	WITH H ₂ ELECTRODE	WITH QUINHYDRONE ELECTRODE
1	5.72	
2	5.67	
3	5.92	
4	5.87	5.83
5	5.85	5.86-5.92*
6	5.87	5.83
7	5.71	
8	{ 5.65	5.65
	{ 5.68	5.77

* Several determinations varied through this range.

gen-ion concentration reported by them for this solution. This fact, combined with the experiments of Coleman for the A. A. C. C., led the associate referee to conclude that the method employed in the collaborative studies of 1926-27 is adapted to the preparation of flour extracts for the determination of hydrogen-ion concentration of flour. It was further concluded that errors that arise in the determination of the hydrogen-ion concentration of flour are not attributable to difficulties inherent in the preparation of the extract, but arise subsequently in the manipulation of the electrodes and the potentiometer. This conclusion seems to be supported by the fact that collaborators report varying results with heavily buffered solutions. Such difficulties probably are general to hydrogen-ion concentration determinations and are quite evidently not confined to flour. These difficulties must be corrected either through an improvement in the technic of the operators, or by some simplification of the electrometric set-up which will render it less sensitive to the varying treatments to which it is subject in different laboratories.

The limited experience with the quinhydrone electrode that has been gained through this series of collaborative studies is hardly sufficient to justify an opinion respecting the propriety of substituting this electrode for the conventional hydrogen electrode. There was a fair agreement among the findings reported by the four collaborators who used the quinhydrone electrode, although two of these collaborators seemingly encountered a greater variation among replicated determinations than resulted from their use of the hydrogen electrode. The associate referee and J. A. Dunn, using various types of electrodes, recently conducted an extensive study of the variability among replicated determinations. It was found that the variability was substantially greater when the hydrogen-ion concentration of a patent flour was determined with the quinhydrone electrode than when hydrogen electrodes were employed.

RECOMMENDATIONS¹.

It is recommended—

(1) That the method presented and adopted as tentative last year be approved as the official method for the determination of the hydrogen-ion concentration of flour. This method has been published².

(2) That attention be given to the possible uses of the quinhydrone and antimony electrodes in the determination of the hydrogen-ion concentration of flour.

REPORT ON GLUTEN IN FLOUR.

By RAYMOND HERTWIG (Hecker H-O Company, Inc., Buffalo, N. Y.),
Associate Referee.

In consequence of the investigations conducted by Dill and Alsberg³ on the effect of various wash solutions on wheat gluten it was recommended at the 1926 convention by the General Referee on Cereal Foods that the present tentative quantitative method for the determination of gluten⁴ be studied and so standardized that concordant and duplicable results may be obtained when the method is used by different analysts and in different laboratories.

The associate referee for 1926 compared results obtained by washing subdivisions of two flour samples with tap water and with 0.1 per cent phosphate solution having a pH of 6.8, respectively. The latter procedure is recommended by Dill and Alsberg. The percentage of dry gluten obtained by washing with tap water was lower than that of the dry gluten washed with phosphate solution for one sample, but it was higher for the second sample. The former associate referee also reported that the phosphate-washed gluten is not so satisfactory as the tap-water-washed gluten for judging gluten quality, and also concluded from the small amount of work done that tap water is still the best wash solution. He considered the determination of gluten quality and gluten quantity by this method of equal importance. No definite recommendations were made.

Before studying this method further it appeared to be desirable to ascertain how cereal chemists evaluate the present method, what information they derive from it, whether it is used extensively, and whether it merits careful study at this time. This information was sought by means of questionnaires and direct discussions, and by reference to appropriate articles appearing in cereal chemistry literature. The opinions

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 37, 85.

² *This Journal*, 1927, 10: 33.

³ *Cereal Chem.*, 1924, 1: 222.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 227.

of a number of prominent cereal chemists, many of whom are active members of the American Association of Cereal Chemists, were obtained in this manner.

The deductions made from the information obtained may be briefly summarized as follows: (1) The gluten method is still used, but not so extensively as formerly; (2) it may give an idea of gluten quality, judged from handling the wheat gluten under fixed conditions of gluten washing, by an experienced and observing operator; (3) the quantities of the gluten ascertained by washing cannot be expressed in definite mathematical terms; and (4) the quantitative results are only approximate.

The quantitative value of the method is limited because a number of factors that affect the results are not easily or practically amenable to standardization. Some of these factors are the temperature, salt content, and hydrogen-ion concentration of the wash solution; the uncertainty of the time and extent of washing; the greater or less vigor and skill of the analyst in manipulating the gluten and in removing non-glutenous substances; and the dispersion and sweeping away of glutenous material. The commercial use of the method is also limited when large numbers of samples must be run, because of the time involved and because the results of different analysts are not comparable.

The general consensus of opinion of cereal chemists seems to be that the chemical protein determination is the best single test for gluten quality. Coleman, Dixon, and Fellows¹ make the following statements in regard to this determination: "Next in order of merit [to the crude protein determination] comes the washed-gluten test. This test gives remarkably good results in the hands of a single operator, but the results obtained are not easily reproducible. This is not true of the crude-protein test". The protein determination is undoubtedly a more reliable index of gluten quantity than the gluten test, and it has the advantages of accuracy and speed.

It is concluded from the information gained that the gluten method should be retained for the present. Later, if it is more extensively utilized it can be readily standardized by development and application of the findings from the gluten studies presented in the literature. In such case the directions for washing should be given in such detail that all analysts would follow the same procedure.

SELECTED REFERENCES.

Recent references of particular note on gluten in the chemical literature are the following:

- (1) DILL and ALSBERG. Some critical considerations of the gluten washing problem. *Cereal Chem.*, 1924, 1: 222.
- (2) KRESS, C. B. Gluten quality. *Cereal Chem.*, 1924, 1: 247.

¹ *J. Agr. Research*, 1927, 34: 262.

- (3) DILL, D. B. The composition of crude gluten. *Cereal Chem.*, 1925, 2: 1.
- (4) SULLIVAN AND NEAR. Chemical constituents which influence gluten quality. *Ind. Eng. Chem.*, 1927, 19: 159.
- (5) COLEMAN, DIXON, and FELLOWS. A comparison of some physical and chemical tests for determining the quality of gluten in wheat and flour. *J. Agr. Research*, 1927, 34: 241.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the method under discussion be designated "crude gluten" instead of "gluten".
- (2) That further study of the method for the determination of gluten be discontinued until circumstances demand its modification and standardization.
- (3) That the parenthetical statement, "results are approximate", be added to the heading, "Quantitative Method.—Tentative", in connection with the tentative method for the determination of gluten.
- (4) That the references given in this report be included in the bibliography of the chapter on cereal foods in *Methods of Analysis*.

REPORT ON DIASTATIC VALUE OF FLOUR.

By E. L. TAGUE² (Kansas State Agricultural College, Manhattan, Kans.),
Associate Referee.

On reviewing the literature bearing upon the determination of the diastatic value of flour it was noted that several investigators had emphasized the need of a uniform substrate. While it is admitted that the raw starch of wheat flour is the natural substrate for the diastatic enzyme, nevertheless starches from different flours vary considerably, and for this reason the results of diastatic activity are not comparable.

Again, it is felt that the present methods might be improved—particularly shortened, but it is admitted that this task is difficult and requires extensive investigation. However, as a start in the right direction, the work for the year was outlined as follows:

A.—Search for a new substrate. This includes (1) the preparation of a uniform starch from wheat flour, if possible; (2) investigation of the utility of starches from other sources; and (3) the possibility of using other related substances which are known to be acted upon by diastase with the production of reducing sugars.

B.—Investigations looking toward the improvement of existing methods.

The following results were obtained:

A (1).—Considerable work was done on the preparation of a uniform product from wheat starch which could be used as a substrate. The resulting products, however, showed no improvement over the raw starch.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 85.

² Presented by F. C. Blanck.

(2) Only two starches from other sources showed improvement in uniformity of quality. These are being fully investigated, and a detailed report will be given later.

(3) Seven substances other than starches were investigated for substrates. Details are being worked out for three of these that give special promise.

REPORT ON STARCH IN FLOUR.

By J. C. PALMER¹ (U. S. Food, Drug and Insecticide Administration, Seattle, Wash.), *Associate Referee*.

The method for the determination of starch outlined by Rask² was submitted to collaborative study again this year. A portion of it had been rewritten. As originally published it appeared to be too long for admission as a tentative method. The sample sent out consisted of a hard-wheat straight-grade flour containing 11.90 per cent moisture (vacuum method).

The suggestion was made that possibly a combination of the Hartmann-Hillig³ method of peptic digestion of starch and the Rask method could be utilized to good advantage in the development of a starch method. Experiments were performed in which the sample was treated with a 1 per cent solution of pepsin and 0.5 per cent hydrochloric acid before the preliminary washings were made. No benefit resulted from this treatment; in fact it slowed up the filtering process considerably. According to Hartmann and Hillig and Bailey⁴, peptic activity and diastatic activity take place under the same conditions. If this is true, there would be no advantage in employing the peptic digestion, since diastatic activity would proceed at the same time and loss of starch would result.

The starch residues from a number of determinations on whole wheat flours were mixed, powdered, and stored in a desiccator, and nitrogen was determined; it amounted to 0.102 per cent, or 0.58 per cent protein.

The hygroscopic nature of precipitated starch was studied. About 0.4 gram of the dry residue was placed in an aluminum moisture dish and heated for 1 hour at 130°C. The dish was uncovered in the oven as well as in the lime desiccator. The cover was placed on the dish and both were weighed. The dish was then replaced in the oven for 1 hour with the cover tilted. At the end of the hour period the cover was forced down, and the dish was removed from the oven to the desiccator and weighed. The contents showed a decrease in weight of 5.5 mg. Owing to its extreme

¹ Presented by F. C. Blanck.

² *This Journal*, 1927, 10: 108.

³ *Ibid.*, 1926, 9: 482.

⁴ *Chemistry of Wheat Flour*, 1925, p. 236.

hygroscopic nature the dried starch had absorbed moisture from the air in the desiccator. Dried flour has a similar characteristic according to Mitchell and Alfend¹.

Very little difference was noted between results obtained by drying the precipitated starch 1 hour or 15 hours, at 130°C. If allowed to stand exposed to room atmospheric conditions 30 minutes, a rapid increase in weight was shown. One conclusion drawn from these experiments was that the drying time of 1½ hours at 130°C. is ample. Another conclusion was that the crucible containing the precipitated starch should be well covered immediately upon removal from the oven and kept covered while in the desiccator and during the operation of weighing.

It has been assumed that the diastatic activity of the flour would be destroyed during the preliminary washings with ether and 70 per cent alcohol. To test this point two samples of the same flour were placed upon filter papers and washed with ether and 70 per cent alcohol, as prescribed in the method. One sample was washed with water, and the determination was completed in the usual manner with no delay; the other sample was kept in a moist condition by the occasional addition of water to the filter during a period of 4 hours, after which the starch determination was completed. The experiment was repeated on the same sample of flour with the following results:

USUAL METHOD	4-HOUR WATER-WASHING PERIOD
<i>per cent</i>	<i>per cent</i>
61.68	60.76
61.48	60.84

The results indicate that the diastatic activity of the sample is not destroyed by the ether and alcohol washings. This point should be kept in mind while making the determination. No delay should be introduced during the water washing because of the diastatic activity and consequent loss of starch.

An experiment was also made to ascertain whether or not any hydrolysis occurs while the sample is in contact with the first few drops of 20.5 per cent hydrochloric acid. Two samples of whole wheat flour were washed in the usual manner with ether, 70 per cent alcohol, and water, and the filter paper containing the charge was put into small beakers, 10 drops of 20.5 per cent hydrochloric acid being added to both. The starch determination was completed immediately on one sample, while the other was allowed to remain in contact with the acid 4 hours after the analysis was completed. The first sample showed a starch content of 61.24 per cent, and the second 61.04 per cent. The conclusion drawn is that the hydrolysis, if any, is small and well within the experimental error of the method. Should it be necessary to introduce any delay

¹ *This Journal*, 1924, 8: 76.

during the operation of the method, it is suggested that it be done at this point; otherwise the determination should be completed as soon as possible.

The method, as modified by the associate referee, has been published¹.

TABLE 1.
Results of collaborators.

ANALYST	STARCH per cent	AVERAGE per cent
M. J. Blish	66.40	
Agricultural Experiment Station	69.88	
Lincoln, Nebr.	64.96	67.16
	67.40	
C. E. Goodrich	71.40	
U. S. Food, Drug and Insecticide Adm.	71.60	
Washington, D. C.		
C. E. Mangels	70.22	
Agricultural College	70.26	70.14
Fargo, N. Dak.	69.94	
M. L. Offutt	70.54	
U. S. Food, Drug and Insecticide Adm.	70.75	
New York, N. Y.	70.67	70.65
J. C. Palmer	70.50	
U. S. Food, Drug and Insecticide Adm.	70.50	70.53
Seattle, Wash.	70.60	
L. G. Petree	68.90	
U. S. Food, Drug and Insecticide Adm.	68.73	68.85
San Francisco, Calif.	68.58*	
	69.17*	
O. S. Rask	70.48	
Johns Hopkins Univ.	70.52	
Baltimore, Md.		
C. B. Morison	70.76	
Institute of Baking	70.02	
Chicago	70.00	
	70.46	70.28
	70.14	
Maximum	71.60	
Minimum	64.96	
Average	69.60	

* 0.5 gram of ignited asbestos added to the alcohol before precipitation of the acid starch.

COMMENTS BY COLLABORATORS.

M. J. Blish.—I am not at all pleased with the lack of concordance in these results, and yet I do not know how I could have followed directions any more closely than I did in each case. Otherwise, I would run some more of the determinations. I am under the impression that I secured much more concordant results last year on the sample of gluten flour sent out by Dr. Rask.

What causes the trouble in this instance, I am unable to say with any assurance. For one thing, I do not have much confidence in the thoroughness with which the flour

¹ *This Journal*, 1928, 11: 37.

is extracted on the filter paper by merely pouring on the various solvents. With flour this may mean that the dextrin is more completely removed in some cases than in others, by the water extraction. Possibly the same is true as to the protein. The final filtration in the Gooch crucible is *very* slow, and I am always suspicious of such filtrations. Under such conditions, if any protein is present, it might be held back on the filter to some extent.

M. L. Offutt.—The method appears to be much quicker and easier than the ones in general use, and it is not particularly difficult after the analyst has used it a few times.

L. G. Petree.—Determinations 3 and 4 were modifications of the proposed method to the extent that 0.5 gram of ignited asbestos was added to the alcohol before precipitation of the acid starch. The presence of asbestos hastened the ultimate filtration, which otherwise was inclined to be slow.

It is probable that the starch could be determined accurately by difference between weights of crucible and contents before and after ashing as the ash of precipitated starch was found to be almost negligible.

C. B. Morison.—One sample of precipitated starch was filtered through an alundum crucible. Filtration was rapid, but the weight of starch was low in comparison to the results submitted. The result was 69.46 per cent.

RECOMMENDATION¹.

It is recommended that the modified Rask method for the determination of starch be adopted as a tentative method.

REPORT ON FLOUR-BLEACHING CHEMICALS.

By G. C. SPENCER (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The work done on the Seidenberg modification² of the tentative method for the determination of chlorine in chlorine-bleached flour suggested a number of interesting problems.

It was found that the direct titration of chlorine, in which chromate was used as an indicator, was not so satisfactory as the Volhard method³. The following points were noted in the use of the Volhard method:

(1) The precipitated silver chloride was removed more satisfactorily by boiling and filtering than by using ether for its coagulation.

(2) The best results were obtained when a standard solution of potassium thiocyanate was used instead of the ammonium salt.

(3) The nitric acid used was rendered free from nitrogen oxides by adding one-fourth its volume of water and boiling until colorless, which condition was preserved when the acid was kept in the dark.

(4) The ferric alum indicator solution was made with a strength of 1 per cent reckoned as $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$; 3 cc. of this solution was taken for a titration.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 86.

² *This Journal*, 1925, 8: 676; 1928, 11: 132.

³ *Ann.*, 1878, 190: 1.

Many of these suggestions were found in articles on the titration of chlorine in potable waters by Stuart¹ and by Shutt and Charlton².

One important phase of manipulation was the method of ashing the extracted fatty matter, but further study will be required before a final recommendation is made. Apparently the ashing can be performed in porcelain crucibles as well as in platinum dishes.

In general, the results obtained by the Rask method³ were about 20 per cent lower for chlorine than those obtained by the Seidenberg modification.

Considerable attention was given to the total chlorine in authentic samples of unbleached flour as well as in chlorine-bleached samples. The ashing was best effected in this case by mixing the flour intimately with a mixture of calcium oxide, magnesium oxide, and magnesium nitrate, and igniting the organic matter after the manner of determining sulfur in coal by the Eschka method⁴. Nickel crucibles were used to good advantage. It will be necessary to do further work to ascertain whether this method can be made applicable. One difficulty may be the procuring of calcium and magnesium oxides that are sufficiently free from chlorine.

Acknowledgments are made to H. M. Joslin for valuable assistance.

It is recommended that further study be made of methods for the determination of chlorine in bleached flour⁵.

No report on methods for bread analysis was given by the associate referee.

REPORT ON EXPERIMENTAL BAKING TESTS.

By M. J. BLISH (Agricultural Experiment Station, Lincoln, Nebr.),
Associate Referee.

This project was actively carried forward during the past year, and substantial progress was made. Detailed reports of the past year's work and findings have been published in the official medium of the American Association of Cereal Chemists⁶, for which the writer is serving as Chairman of the Committee on Standardization of the Experimental Baking Test. These reports were prepared for the recent annual meeting of that association, which happens to precede the meet-

¹ *J. Am. Chem. Soc.*, 1911, 33: 1344.

² *Trans. Roy. Soc. Can.*, 1905.

³ *This Journal*, 1922, 6: 68.

⁴ *Z. anal. Chem.*, 1878, 17: 497.

⁵ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 86.

⁶ *Cereal Chem.*, 1927, 4: 299.

ing of the Association of Official Agricultural Chemists. For this reason the statement herewith submitted may properly be confined to a brief and general account of the present status of the project.

Detailed surveys, inquiries, inspections, questionnaires, etc., indicated, among other things, that the great majority of cereal chemists regard the baking test as the most important of the various laboratory flour tests.

This test, however, is *not* generally conducted according to the definite well-recognized principles that should govern the scientific testing of a material. From this viewpoint the *type* of procedure that has been used in most, though not all, cereal-testing laboratories is deserving of adverse criticism on the ground that it involves variables other than the material that is to be tested. The sort of manipulation too frequently employed depends to a large degree upon the skill and interpretative judgment of the operator. Any test of this kind falls into the category of art rather than science, and obviously it cannot lend itself to the standardization that is desired.

There is a type of laboratory baking test that seeks merely to test the material, i. e. flour, and in which there is a deliberate attempt to eliminate all other variables. Here all flours (at least within a given class) are subjected to precisely the same treatment and environment. Artistry and manipulative skill are eliminated to the greatest possible degree. During the past year the committee of the American Association of Cereal Chemists, both collectively and individually, made inquiry into the possibilities of this procedure, which may be referred to as the "fixed" type, and subjected it to critical laboratory study.

In such a test, under proper environmental control, no unusual skill or artistry is required insofar as actual performance is concerned. Duplicate tests on individual flours check satisfactorily, whether made by the same operator or by different operators, provided reasonable care in manipulation and accurate environmental control, especially as to temperature, are maintained. Differences in flour characteristics may also be readily distinguished, and with a moderate amount of experience these differences may be translated into terms of commercial utility of the flour. Blish and Sandstedt¹ have recently published a study of certain factors to be taken into account in the interpretation of baking tests conducted according to the "fixed" principle.

Reference has been made to the detailed report of the committee, which has reached the general conclusions herewith briefly presented. The fixed type of method is endorsed by the committee, and advocated as the only logical procedure upon which a standard scientific laboratory baking test may be founded. This report has been accepted by the American Association of Cereal Chemists. Insofar as that organization

¹ *Cereal Chem.*, 1927, 4: 291.

is concerned, the project now becomes a matter of deciding upon the actual details of the test itself. These details involve a large number of items that are being made objects of laboratory investigation. It is confidently expected that by another year a tentative method will be ready for collaborative study¹.

REPORT ON FAT, LIPOIDS AND LIPOID PHOSPHORIC ACID (P_2O_5), WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL, AND UNSAPONIFIABLE MATTER IN ALIMENTARY PASTES, AND UNSAPONIFIABLE MATTER IN FLOUR.

By SAMUEL ALFEND (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.), *Associate Referee*.

The methods sent out for collaborative study this year required careful analytical work, and the associate referee wishes to express his appreciation of the services given by the following collaborators, who submitted reports:

L. H. Bailey, Food Control Laboratory, Washington, D. C., and the following from the U. S. Food, Drug and Insecticide Administration laboratories: J. H. Bornmann, Chicago, Ill.; L. H. Chernoff, Denver, Colo.; J. Fitelson, Philadelphia, Pa.; L. A. Salinger, Savannah, Ga.; W. C. Woodfin, Baltimore, Md.

Samples of patent flour (A), water noodles (B), and egg noodles (C) were prepared, and subdivisions were sent out with directions for analysis. The directions submitted to the collaborators follow:

FAT.

(To be run on Samples B and C.)

Method 1. Direct Extraction.—Determine as directed in *Methods of Analysis A. O. A. C.*, 1925, p. 225, 3 (p. 117, 13).

Method 2. Acid Hydrolysis.—Determine according to the tentative method, *This Journal*, 1926, 9: 41, except that the sample should be weighed directly into a Mojonnier tube, and only enough alcohol should be added to bring the liquid to the mark, instead of 10 cc.

UNSAPONIFIABLE MATTER.

(To be run on Samples A, B, and C.)

Method 1. Modified Kerr-Sorber Method.—Extract the lipoids from 5 grams of sample according to the tentative method, *This Journal*, 1926, 9: 40. To the crude lipoids, add 30 cc. of alcohol and 3 cc. of concentrated potassium hydroxide (1 + 1), and proceed according to the modified Kerr-Sorber method, *This Journal*, 1925, 8: 441.

Method 2. F. A. C. Method.—Determine the unsaponifiable matter in the crude lipoids, obtained as in Method 1 above, by the F. A. C. Method, *This Journal*, 1926, 9: 45.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 86.

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

*Method 1. (To be run on Samples B and C).—*Proceed according to the directions given for flour, *This Journal*, 1926, 9: 40.

*Method 2. (To be run on Sample C).—*Place 20 grams of the sample in a 200 cc. nursing bottle, add 100 cc. of 1.2 per cent sodium chloride solution from a pipet, shake the bottle vigorously to prevent lumping of the sample, and add exactly 100 cc. more of the sodium chloride solution. Shake the stoppered bottle in a mechanical shaker or by hand for 30 minutes. (The temperature of the water should not exceed 30°C.) Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. (The filtrate should be practically clear.) Pipet 50 cc. of the filtrate into a 200 cc. nursing bottle, add 0.2 gram of asbestos, shake, and with constant agitation add 35 cc. of 95 per cent alcohol. Let stand overnight, then centrifugalize to pack the precipitate and asbestos. If the liquid is perfectly clear, pour it off, and wash with two 20 cc. portions of 40 per cent alcohol, in each case shaking, centrifugalizing, and decanting. If the liquid is not free of suspended matter, filter through a thin asbestos pad (0.1–0.15 gram) in a Gooch crucible, using light suction. Filter the subsequent washings also. Transfer the precipitate and asbestos in the nursing bottle to a Kjeldahl flask with the aid of a stream of water, add to it the mat in the Gooch crucible, and determine the nitrogen as directed on p. 8, 22 (*Methods of Analysis*, A. O. A. C., 1925), collecting the ammonia in 10 cc. of 0.1 *N* acid. Run a blank determination on the reagents, using as nearly as possible the same quantity of asbestos.

*Method 3. (To be run on Sample C).—*Proceed as in Method 2 through the filtration of the extracted sample from the dilute salt solution. Then determine nitrogen in 50 cc. of the filtrate, as directed on p. 8, 22. Distil the ammonia into 20 cc. of 0.1 *N* acid. Run a blank on the reagents. Pipet off 100 cc. of the above filtrate into a 200 cc. volumetric flask. Add 70 cc. of 95 per cent alcohol by volume. Mix carefully to avoid foaming, cool to room temperature, and make up to volume with 40 per cent alcohol. Shake well and let stand overnight to allow complete precipitation of the albumin. Pipet off the supernatant solution and filter through an asbestos pad in a Hirsch funnel or Gooch crucible, using light suction. Determine nitrogen in 75 cc. of the filtrate as above. Distil the ammonia into 10 cc. of 0.1 *N* acid. Run blank determinations on the reagents. Multiply the result for nitrogen in 75 cc. of the 40 per cent alcohol filtrate by four-thirds, and subtract the product from the nitrogen contained in 50 cc. of the water extract, to determine the quantity of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in 50 cc. of the salt solution extract.

LIPOIDS AND LIPOID PHOSPHORIC ACID (P_2O_5).

(To be run on Samples B and C.)

Proceed as for flour, *This Journal*, 1926, 9: 40.

COMMENTS OF COLLABORATORS.**FAT.**

L. H. Bailey.—The ether extraction method is not satisfactory for products of this type. I have no unfavorable comment to make on the acid hydrolysis method.

J. H. Bornmann.—No difficulty in manipulation was experienced with either the direct extraction or the acid hydrolysis method for fat. It is quite evident that direct extraction does not remove the fat from a product of the nature of noodles. If the material were ground extremely fine, it is probable that the ether would extract the fat; however this extraction is accomplished much more easily by the acid hydrolysis method.

J. Fitelson.—Röhrig tubes were used in the absence of Mojonnier tubes. It is quite evident that much time in handling would be saved by weighing the sample directly into the Mojonnier tube. Consistently higher results (1.20 per cent) on samples A and B were secured by the acid hydrolysis method, which method is also much more rapid than the direct extraction method. The direct extraction method requires less attention and handling than the acid hydrolysis method. However, in view of the accuracy and rapidity of the latter method, the acid hydrolysis method seems preferable.

LIPOIDS AND LIPOID PHOSPHORIC ACID (P_2O_5).

J. H. Bornmann.—I have used the methods for some time and have found them quite satisfactory.

J. Fitelson.—A satisfactory method except that it does not specify running a blank. A blank of 0.0030 gram was secured on the reagents (mostly from the ether), which introduces an error of 0.06 per cent. This blank was not used in calculating the lipoids.

UNSAAPONIFIABLE MATTER.

L. H. Bailey.—The modified Kerr-Sorber method is objectionable for the reason that one is likely to obtain an emulsion which is difficult to break. This tendency is not nearly so pronounced with the F. A. C. method, and the two methods seem to give practically the same results. For this reason, preference is given to the F. A. C. method.

J. H. Bornmann.—So far as results are concerned, there seems to be little choice between the modified Kerr-Sorber method and the F. A. C. method. I prefer the former; however, if it were possible to dispense with separatory funnels entirely in the F. A. C. method, I should consider that a strong point in its favor. Large separatory funnels which do not leak are not plentiful in the ordinary laboratory. The extraction cylinder is a simpler piece of apparatus and costs less than one-half as much as the separatory funnel. One undesirable feature about the F. A. C. method is the large number of extractions.

J. Fitelson.—The modified Kerr-Sorber method is rapid and quite satisfactory. In the F. A. C. method an ordinary 250 cc. glass-stoppered graduated cylinder was used instead of the specified cylinder. Much difficulty was experienced in the use of a glass siphon to draw off the ether extract as it is a cumbersome piece of apparatus and requires some experience in order to use it. It is necessary to use a slender glass siphon with the petroleum ether, and these siphons are fragile and subject to breakage. A direct extraction of the liquid in a separatory funnel with seven successive portions of petroleum ether, although tedious and time-consuming, would eliminate the use of the cylinder-siphon arrangement with its many loopholes for errors. However, the modified Kerr-Sorber method appears to have a great advantage over the F. A. C. method in regard to the time consumed and accuracy of the method. The modified Kerr-Sorber method is much more rapid, is less tedious since there are less extractions to be made, and allows less chance for error due to manipulation.

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

L. H. Bailey.—Methods 2 and 3 seemed to give higher results than Method 1, Method 3 being the most complicated. Method 2 is preferred provided the precipitate obtained by this method contains the material sought.

J. H. Bornmann.—Method 3 appears preferable because of the ease of determining nitrogen on a solution rather than on a solid mixed with asbestos. The filtration is not so troublesome because it is only necessary to obtain a sufficient volume of filtrate for analysis. The question of washing a precipitate or of transferring the mass from the filter to the flask does not enter into this method.

J. Fitelson.—

Method 1.—Filtering the precipitated protein through an asbestos pad proved to be an extremely slow process, so a qualitative filter paper on a Hirsch funnel was substi-

tuted, and rapid filtration resulted. Blanks, in which pads were used in some cases and filter paper in others, gave identical results.

Method 2.—The comments on the manipulation are the same as in Method 1, except that some time is saved by using the 1.2 per cent salt solution instead of weighing out 1.2 grams for each determination, as in Method 1. Otherwise both methods are quite satisfactory, each giving consistent results.

Method 3.—No advantage over Method 2 is offered by Method 3. It offers more chances for inaccuracies, one source being the concentration of the alcoholic solutions during filtration with suction. More time and material are also consumed by this method. Methods 2 and 3 give higher results than Method 1, explicable only by the supposition that more protein is soluble in 1.2 per cent salt solution than in water, so that although each method yields consistent results, these results are to be interpreted according to the method used. The time saved by extracting directly with the 1.2 per cent salt solution would favor Method 2 over Method 1. It is also noted that Method 1 specifies collecting the ammonia in 10 cc. of 0.1 *N* acid, as does Method 2. Method 3 specifies the use of 20 cc. of 0.1 *N* acid before the precipitation of the proteins. Although these quantities are sufficient for water noodles, they are insufficient for egg noodles; therefore it is suggested that where the method reads 10 cc., 20 cc. should be substituted, and where it reads 20 cc., 30 cc. should be specified.

DISCUSSION.

FAT.

The acid hydrolysis method has previously been shown to be quite satisfactory for flour and for alimentary pastes, and the results obtained in this year's work, though showing a somewhat greater variation among analysts than has been found heretofore, are fairly satisfactory. It seems worth while, however, to change the method slightly to allow weighing the sample directly into the extraction tube.

LIPIDS AND LIPOID PHOSPHORIC ACID (P_2O_5).

The collaborators were able to check themselves closely, but the agreement among collaborators is not so close as might be desired. The results for lipoids on Sample C are similar to those obtained in the collaborative work on flour two years ago. They may be placed in two groups, the results in each group checking well but the variation between the two groups being appreciable. The blank on the reagents is not large enough to account for the variation. It is believed that the method is the best available and that it has been tested sufficiently to warrant its recommendation as an official method.

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

The official method for this determination in flour requires the filtration of the precipitated proteins through an asbestos mat, and the thorough washing of the precipitate. This process is difficult and time-consuming when the quantity of precipitate is considerable, as it is in egg noodles. To obviate this difficulty, R. Hertwig¹ suggested centrifugalizing the precipitated proteins and decanting off the supernatant liquid. He

¹ *This Journal*, 1923, 7: 84.

also suggested a special form of tapered tube to hold the precipitate. The associate referee found an ordinary nursing bottle to be quite satisfactory. Palmer attempted to avoid the complete filtration and washing by determining nitrogen in the solution before precipitation of the alcohol-insoluble protein, and in the filtrate after precipitation. As it is necessary, in the latter method, to filter only enough solution to obtain an aliquot for determining nitrogen, the filtration may be done quickly and easily.

Hertwig objected to Palmer's method because it requires two nitrogen determinations, and thus increases the chance for error. The collaborative work, however, shows that the two methods give practically identical results and that the variation is no greater by the indirect method than by the direct method. Of the three collaborators who commented on the relative merits of Methods 2 and 3, two favored Method 2 and one preferred Method 3. The associate referee is inclined to favor the indirect method, but he is of the opinion that a more extended comparison of the two methods should be made. In his capacity as Associate Referee on Eggs and Egg Products it will be necessary for him to examine these methods for eggs and to submit them for collaborative work. Since the same factors are involved in both classes of products, and any unnecessary demand on the collaborators for making these tedious analyses should be avoided, it is believed that pending the outcome of this work on eggs no further collaborative work should be done. Except for the use of 1.2 per cent salt solution for the initial extraction of protein, either Method 2 or Method 3 is worthy of inclusion as a tentative method at the present time. The salt solution was suggested by Palmer as a better solvent for proteins than water, and, as several of the collaborators have noted, it saves time by avoiding the weighing out of salt before the precipitation with alcohol. The collaborative work shows that the salt solution extracts considerably more alcohol-insoluble protein than does pure water. Since the method for flour specifies "water-soluble" nitrogen, and all the authentic data bearing on the differentiation of egg yolk products from whole egg products, for which these methods were devised, are based on the water-soluble nitrogen, the salt solution should be replaced by water in the method as applied to alimentary pastes.

UNSAPONIFIABLE MATTER.

Of the four collaborators who submitted reports on unsaponifiable matter, one favored the F. A. C. method, one favored the modified Kerr-Sorber method, one had a preference for the Kerr-Sorber method but acknowledged certain features in favor of the F. A. C. method, and one indicated no preference. The results obtained by the two methods are nearly the same, but those obtained by the F. A. C. method are slightly

TABLE 1.
Collaborative results on fat.

COLLABORATOR	DIRECT EXTRACTION		ACID HYDROLYSIS	
	B	C	B	C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Bailey	0.25	2.38	1.45	3.73
	0.23	2.20	1.47	3.78
		2.57		
Bornmann	0.21	2.52	1.60	3.97
	0.21	2.53	1.53	4.01
Chernoff	0.36	2.95	1.54	3.79
Fitelson	0.35	2.58	1.55	3.77
	0.33	2.58	1.55	3.78
Salinger	0.20	2.65	1.53	3.50
	0.26	2.65	1.47	3.56
Woodfin	0.43	2.56	1.49	3.40
	0.51	2.54	1.42	3.39

TABLE 2.
Collaborative results on lipoids and lipid phosphoric acid (P_2O_5).

COLLABORATOR	LIPOIDS		LIPOIDS P_2O_5	
	B	C	B	C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Bailey	1.95	4.08		
	2.14	4.00		
Bornmann	2.42	4.68	0.040	0.108
	2.39	4.65	0.050	0.110
Chernoff	1.48	4.05	0.051	0.116
Fitelson	1.79	4.13	0.044	0.096
	1.82	4.13	0.047	0.098
Salinger	1.86	4.45	0.047	0.112
	1.89	4.46	0.047	0.111
Woodfin	1.27	3.95	0.028	0.0905
	1.50	3.97	0.033	0.0908

higher, in general. The variation is about the same in the two methods. It is noted in some cases that the variation in each method exceeds the minimum value for unsaponifiable matter, which is most unsatisfactory. The differences of opinion among the collaborators appear to be based on considerations of speed and convenience.

TABLE 3.

Collaborative results on water-soluble protein-nitrogen precipitable by 40 per cent alcohol.

COLLABORATOR	METHOD 1		METHOD 2	METHOD 3
	B	C	C	C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Bailey	0.017	0.191	0.28	0.265
	0.020	0.193	0.31	0.276
Bornmann	0.025	0.202	0.247	0.316
	0.025	0.202	0.252	0.289
Fitelson	0.030	0.195	0.283	0.282
	0.029	0.191	0.283	0.272
Salinger	0.03	0.20	0.26	0.27
	0.03	0.20	0.27	0.27
Woodfin	0.042	0.36	0.27	0.244
	0.050	0.38	0.25	

TABLE 4.

Collaborative results on unsaponifiable matter.

COLLABORATOR	MODIFIED KERR-SORBER METHOD			F. A. C. METHOD		
	A	B	C	A	B	C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Bailey	0.13	0.17	0.31	0.19	0.17	0.35
	0.13	0.19	0.26	0.17	0.17	0.40
Bornmann	0.15	0.12	0.26	0.14	0.13	0.25
	0.12	0.10	0.25	0.11	0.13	0.23
Fitelson	0.23	0.22	0.45	0.24	0.16	0.41
Woodfin	0.09	0.08	0.28	0.12	0.078	0.31
	0.11	0.11	0.28	0.13	0.061	0.45
Average	0.14	0.14	0.30	0.16	0.13	0.34
Variation	0.14	0.14	0.20	0.12	0.11	0.22

Last year the associate referee recommended the adoption of the F. A. C. method and gave his reasons for that recommendation. The general referee disapproved of this recommendation and recommended that the Kerr-Sorber method be adopted as tentative, because the collaborative results obtained by the latter method were appreciably more uniform than those obtained by the F. A. C. method. This year's work does not show that the modified Kerr-Sorber method gives more uniform results than the F. A. C. method.

Since the reasons advanced by the associate referee for favoring the F. A. C. method are still valid, and since in this year's work the modified

Kerr-Sorber method shows no advantage over the F. A. C. method in respect to uniformity of results, the associate referee is again recommending the F. A. C. method for adoption as a tentative method.

RECOMMENDATIONS¹.

It is recommended—

(1) That the tentative acid hydrolysis method for the determination of fat in alimentary pastes, with the slight change described in this report, be adopted as official.

(2) That the tentative method for the determination of lipoids and lipid phosphoric acid (P_2O_5) be adopted as official for alimentary pastes.

(3) That the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol be subjected to further study.

(4) That the tentative modified Kerr-Sorber method for the determination of unsaponifiable matter be dropped.

(5) That the F. A. C. method for the determination of unsaponifiable matter be adopted as a tentative method for flour and alimentary pastes.

No report on moisture in alimentary pastes was given by the associate referee.

ADDRESS BY R. W. DUNLAP, ASSISTANT SECRETARY OF AGRICULTURE.

MR. PRESIDENT AND AGRICULTURAL CHEMISTS. I do not come before you at this time with the intention of making any extended remarks.

The United States Department of Agriculture is appreciative of the fine, friendly inter-relationship which exists between Federal chemists and industrial chemists and also among the officials of the various States and political subdivisions. Your particular groups are functioning in the process of securing the benefits of chemical research and scientific discoveries for the consumer, or the people, under regulatory measures enacted and administered in behalf of the people. In many instances the people have but a slight conception of all the safeguards that are provided to assure their health and happiness, and that further assure true value in the purchase of life's necessities for which the greater part of their earnings are expended.

The experimentations and developments of chemists and scientists are based upon principles of truth, and no progress can be made in perfecting scientific development of facts upon anything but the absolute truth of known principles. In such a sphere of official duties you are the

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 38, 87.

agencies to perpetuate these truths and to introduce them into the business practices of manufacturing and merchandising. You do this under certain standards and tolerances, of course, but to the end that dollar's worth for dollar of not less than standard quality and constituency will reach the consumer through the channels of trade.

Because your vocation is highly specialized and your duties are of detailed nature, your accomplishments may be unobserved in the everyday life of our millions of people. I shall not attempt to utter the deserving mention that might be made of your services nor could I discourse intelligently on the technic of your duties. But more and more and in greater numbers we have come to realize the benefits we receive through scientific research work and the proper administration of the laws under which this research advantage is transmitted to the people. Your duties in requiring compliance with the rules and laws enacted and promulgated to guarantee these benefits to the trade and to the consumer in our commercial world are well performed.

If it were not for your vigilance and keen ability to discover spurious goods it might be quite common for us to buy food or drugs of inferior if not injurious quality—synthetic milk, for example, without the proper component of yellow skum, or high-powered drug and opiate pellets consisting of colored or brown sugar, or even worse than that.

One particularly outstanding accomplishment or service you have rendered is the compilation of the book of methods of analysis. The Department has a prime interest in the continuation of the current activities of your members in this service.

Glancing through the 32 chapters contained in *Methods of Analysis of the Association of Official Agricultural Chemists*, one is really amazed at the tremendous amount of work that has been done in preparing this unusual and epoch-making publication in this particular field of chemistry. When we realize that all this work has been done voluntarily, without any thought of profit on the part of men and women engaged in public service, we are struck with the splendid service that has been rendered. An examination of the methods that have been developed for the examination of soils, plants, tanning materials, leathers, sugar and sugar products, cereal foods, canned foods, and meat and meat products creates appreciation of the fact that here is the very foundation of successful research work in those activities that are peculiarly within the field of the United States Department of Agriculture.

From an administrative standpoint, the Department feels that one of its most profitable investments is the small amount of funds that is annually devoted to the Department's activities in cooperating with you in this development. I am told that the book of methods of your association has become a sort of chemist's bible for all those who are interested in the large field generally referred to as "agricultural chemistry",

and I understand that this work so auspiciously started and so splendidly carried on during a period of 40 years has entailed a great deal of self-sacrifice on the part of many of your members.

The research work of the Department, as well as of the industries that may be based on the fundamental knowledge of the chemical constituents of plant and animal material, is indeed dependent upon accurate information, such as is obtained through the processes and technic outlined in your *Methods of Analysis*. It is a monument in which each one of you may feel a very personal and distinct pride.

The identity of great scientists may be little known, and the individuals composing this association are of that type of modest beings who do very little self-advertising; nevertheless I am certain your efforts are being appreciated by the public. It is the desire of the Department of Agriculture to convey a frank expression of appreciation of your work and to assure you that it shall be our purpose to continue the fine co-operative spirit that has always existed. It has afforded a most enjoyable collaboration for all of us. As one public servant to another may I express the appreciation of your work which I know exists generally in every instance where the public have come to realize the advantages they receive through your faithful services.

No report on specific gravity and alcohol was given by the referee.

REPORT ON VINEGARS.

By J. O. CLARKE, *Referee*, and J. FELDBAUM (U. S. Food, Drug and Insecticide Administration, New York, N. Y.).

Last year the referee suggested that methods for total and for soluble ash be further studied and that the subject of phosphoric acid in vinegar be studied with a view to the possible substitution of a method for the determination of total phosphoric acid for the present methods for soluble and insoluble phosphoric acid. Also it was recommended that the methods for glycerol, sulfates, and polarization be given attention. This report will be confined to studies made in the writers' laboratory on ash and phosphoric acid.

TOTAL ASH.

Total ash was determined on a sample of vinegar by both the official Methods, A and B¹. Studies were made of the effect of the temperature

¹ *Methods of Analysis*, A. O. A. C., 1925, 325.

of incineration, the quantity of sample used, the effect of cooling outside the desiccator and of spreading agents.

Quantities of 100 cc., 50 cc., 25 cc., and 10 cc. were incinerated by Methods A and B at the lowest (barely visible) dark red heat, at medium heat, and at very bright red heat,

Comparative results on total ash by Methods A and B at lowest red heat follow:

SIZE OF SAMPLE	METHOD A	METHOD B
cc.	gram per 100 cc.	gram per 100 cc.
100	0.26	0.28
100	0.27	0.27
100	0.27	0.27
100	0.26	0.26
100	0.26	0.27
50	0.26	0.27
50	0.27	0.28
50	0.26	0.28
25	0.28	0.28
25	0.29	0.28
25	0.23	0.30
25	0.27	0.30
10	0.25	0.29
10	0.33	0.32
10	0.29	0.31

The above tabulation shows that Method A gives slightly lower results than Method B, the average for fifteen determinations on the same sample by Method A being 0.27 and by Method B, 0.284 gram per 100 cc. The variation in the results by Method A are greater than by Method B, the highest and lowest in Method A being, respectively, 0.33 and 0.23, and in Method B, 0.32 and 0.26. It appears from the tabulation that closer checks are obtained when a larger sample is taken.

Comparative results on water-soluble ash by Methods A and B at lowest red heat follow:

SIZE OF SAMPLE	METHOD A	METHOD B
cc.	gram per 100 cc.	gram per 100 cc.
100	0.22	0.24
100	0.23	0.23
100	0.24	0.23
100	0.23	0.23
100	0.22	0.24
50	0.23	0.23
50	0.23	0.24
50	0.23	0.25
25	0.24	0.24
25	0.25	0.26
25	0.20	0.27
10	0.20	0.25
10	0.28	0.28
10	0.27	0.28

The remarks made on total ash in the previous table also pertain to the results in the above tabulation.

Comparative results on total ash incinerated at lowest (barely visible) dark-red heat, at medium heat, and, at very bright red heat are as follows:

SIZE OF SAMPLE	LOWEST RED HEAT	MEDIUM RED HEAT	BRIGHT RED HEAT
cc.	gram per 100 cc.	gram per 100 cc.	gram per 100 cc.
100	0.27	0.26	0.06
100	0.26	0.26	0.06
100	0.27	0.26	
100	0.27	0.25	
50	0.28	0.26	0.05
50	0.26	0.25	0.08
25	0.30	0.26	0.07
25	0.23	0.22	0.05
10	0.31	0.27	0.07
10	0.29	0.27	0.06

It appears from the above table that there is but a small loss in total ash when the heat of incineration is increased from the lowest redness to a medium red heat, but that there is a tremendous loss when the temperature is increased to a rather bright red heat. Thus the loss at medium red heat is 0.018 gram per 100 cc., or 7 per cent of the total ash as an average of ten determinations, whereas the loss at a high bright red heat is more than 75 per cent of the total ash.

For the purpose of determining the error due to faulty desiccation or cooling the ash in the open air, one dish containing 25 cc. of vinegar was ashed by Method B at very low redness, cooled in a desiccator containing fresh sulfuric acid, and weighed rapidly. It was then permitted to remain on the balance and weighed every half hour for 2 hours. After standing on the open balance the first half hour there was a gain of 9.5 mg.; after that there was no appreciable gain. The difference in the ash after being cooled in the desiccator and rapidly weighed and after being permitted to remain in the air a half hour was 0.038 gram per 100 cc., the first figure being 0.272 gram and the second figure being 0.310 gram.

In order to facilitate the ashing of the vinegar, powdered alundum was added, 1 gram to 25 cc. The alundum was of no value in the direct incineration of the vinegar to a gray ash and when Methods A and B were used the results were slightly low (0.02 gram per 100 cc. or about 4 per cent low).

The use of commercial granulated sugar was found to be of value in the direct ashing of vinegar at a low temperature. The addition of 1 gram of granulated sugar to 25 cc. of vinegar produced a gray ash after 3-4 hours incineration at low redness. The granulated sugar was found to have no weighable ash per 5 grams of material. The following total ash and water-soluble ash results were obtained on the addition of granulated sugar and incineration:

SIZE OF SAMPLE	SUGAR USED	TOTAL ASH		WATER-SOLUBLE ASH	
		Low redness	Medium redness	Low redness	Medium redness
cc.	gram	per cent	per cent	per cent	per cent
25	1	0.28	0.28	0.25	0.24
25	1	0.31	0.28	0.28	0.24
25	1	0.30	0.28	0.27	0.24
25	2	0.28	0.25	0.24	0.22
50	5	0.26	0.27	0.23	0.23
25	1	0.28	0.27	0.24	0.24
25	1	0.28	0.25

The results for total and water-soluble ash compare favorably with the results obtained by Methods A and B. It was found that the vinegar can be quickly incinerated to a gray ash if the vinegar and 1 gram of sugar are first charred on a burner and wetted down slightly, the water evaporated on a steam bath, and the dish placed in the muffle. It required less than an hour to burn to a gray ash in this manner.

PHOSPHORIC ACID.

Soluble and insoluble phosphoric acid were determined on the ash reported in Table 3. At higher temperatures of ashing there is uniformly a greater proportion of soluble phosphoric acid, although the sum total of soluble and insoluble phosphoric acid is not greatly affected.

SIZE OF SAMPLE	LOWEST RED HEAT			MEDIUM RED HEAT		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total
cc.	mg. per 100 cc.	mg. per 100 cc.		mg. per 100 cc.	mg. per 100 cc.	
100	9.6	8.4	18.0	10.1	7.8	17.9
100	9.8	8.4	18.2	12.3	6.2	18.5
100	9.8	8.7	18.5	12.0	5.9	17.9
100	9.7	8.7	18.4	12.6	5.1	17.7
50	9.6	8.3	17.9	12.5	5.1	17.6
50	9.5	9.1	18.6	13.1	6.0	19.1
25	9.7	8.5	18.2	12.9	5.0	17.9
25	9.3	9.4	18.7	11.6	6.7	18.3
10	9.0	8.5	17.5	12.0	6.5	18.5
10	8.7	9.8	18.5	11.7	6.8	18.5

The results shown for phosphoric acid confirm those reported last year in that even a small increase in the temperature of ashing changes the ratio between soluble and insoluble phosphoric acid, although no change is noted in total phosphoric acid. Accordingly, separate determinations for soluble and insoluble phosphoric acid should be omitted, and the method for total phosphoric acid substituted therefor.

RECOMMENDATIONS¹.

It is recommended—

(1) That methods for the determination of total and soluble ash involving the use of substances to assist in the formation of a porous ash material, such as sugar, be further studied.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 82.

(2) That Methods 8 and 9¹, for the determination of soluble and insoluble phosphoric acid, be further studied.

(3) That methods for the determination of glycerol and sulfates and for the polarization of vinegars be further studied.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES.

By J. W. SALE² (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

Analysis of Non-alcoholic Flavors.

In compliance with last year's recommendation³ new analytical data were obtained on the determination of certain essential oils in non-alcoholic flavors by the steam distillation method⁴. A detailed description of this method has been published⁵.

The method was tested in 1925 by two collaborators; this year the analytical work was conducted by a collaborator who had had no previous experience with the method. The results obtained are given in Table 1, and these data are comparable with those in Table 3 in the report for 1925⁶.

DISCUSSION OF DATA IN TABLE 1.

The data in Table 1 show that the maximum percentage error on 16 samples (experiments 51-66 inclusive) of orange and lemon oils dissolved in corn and cottonseed oils was -2 per cent, the average being only 0.5 per cent. Corresponding figures obtained in 1925 on 30 samples (experiments 6-35 inclusive) by collaborators using the factor 0.90 for lemon oil were +7 per cent and 1.9 per cent, respectively. The maximum variation on one sample of +7 per cent is regarded as exceptionally large in the light of the data obtained on the other samples of lemon and orange oils in vegetable oils.

The maximum percentage error on 15 samples (experiments 67-81 inclusive) of oil of limes in corn and cottonseed oils was ± 3 per cent, the average being only 0.7 per cent. Oil of limes was not determined in 1925.

The maximum percentage error on 10 samples (experiments 82-91 inclusive) of oil of nutmeg in corn and cottonseed oils was ± 3 per cent, the average being 2 per cent. Oil of nutmeg was not determined in 1925.

The maximum percentage error on 10 samples (experiments 92-101 inclusive) of peppermint oil in corn and cottonseed oils was -7 per cent, the average being 3.3 per cent.

¹ *Methods of Analysis*, A. O. A. C., 1925, 325.

² Presented by J. B. Wilson.

³ *This Journal*, 1927, 10: 76.

⁴ *Ibid.*, 1926, 9: 450.

⁵ *Ibid.*, 1928, 11: 45.

⁶ *This Journal*, 1926, 9: 452.

TABLE 1.

Results of determination of essential oils in corn oil and cottonseed oil by steam distillation.*

EXPERIMENT NO.	ESSENTIAL OIL PRESENT, BY VOLUME per cent	ESSENTIAL OIL FOUND, BY VOLUME† per cent	ESSENTIAL OIL FOUND, BY VOLUME CORRECTED‡ per cent	ERROR¶ per cent
Expressed orange oil in corn oil.				
51	5.00	4.65	4.89	-2
52	5.00	4.80	5.05	+1
53	5.00	4.70	4.95	-1
Expressed lemon oil in corn oil.				
54	5.00	4.55	5.06	+1
55	5.00	4.50	5.00	0
56	5.00	4.50	5.00	0
57	5.00	4.55	5.06	+1
58	5.00	4.50	5.00	0
59	5.00	4.50	5.00	0
60	5.00	4.50	5.00	0
61	5.00	4.55	5.06	+1
Expressed lemon oil in cottonseed oil.				
62	5.00	4.50	5.00	0
63	5.00	4.50	5.00	0
64	5.00	4.50	5.00	0
65	5.00	4.45	4.94	-1
66	5.00	4.50	5.00	0
Distilled oil of limes in corn oil.				
67	5.00	3.95	5.00	0
68	5.00	3.95	5.00	0
69	5.00	3.95	5.00	0
70	5.00	4.00	5.06	+1
Expressed oil of limes in corn oil.				
71	5.00	3.85	5.00	0
72	5.00	3.95	5.13	+3
73	5.00	3.75	4.87	-3
74	5.00	3.85	5.00	0
Distilled oil of limes in cottonseed oil.				
75	5.00	4.00	5.06	+1
76	5.00	3.98	5.04	+1
77	5.00	3.95	5.00	0
78	5.00	3.98	5.04	+1
79	5.00	3.95	5.00	0
Expressed oil of limes in cottonseed oil.				
80	5.00	3.85	5.00	0
81	5.00	3.90	5.07	+1
Nutmeg oil in corn oil.				
82	2.00	1.45	1.93	-3
83	2.00	1.55	2.07	+3
84	2.00	1.55	2.07	+3
85	2.00	1.45	1.93	-3
86	2.00	1.45	1.93	-3
Nutmeg oil in cottonseed oil.				
87	2.00	1.45	1.93	-3
88	2.00	1.50	2.00	0
89	2.00	1.45	1.93	-3
90	2.00	1.50	2.00	0
91	2.00	1.50	2.00	0
Peppermint oil in corn oil.				
92	3.00	1.70	2.79	-7
93	3.00	1.90	3.11	+4
94	3.00	1.90	3.11	+4
95	3.00	1.75	2.87	-4
96	3.00	1.90	3.11	+4
Peppermint oil in cottonseed oil.				
97	3.00	1.85	3.03	+1
98	3.00	1.85	3.03	+1
99	3.00	1.80	2.95	-2
100	3.00	1.90	3.11	+4
101	3.00	1.80	2.95	-2

* These data are a continuation of those in Table 3, *This Journal*, 1926, 9: 452. Analyses were made by C. H. Badger, Food, Drug and Insecticide Administration, Washington, D. C.

† 100 cc. of the sample was taken, and 200 cc. of distillate was obtained. The essential oils were recovered in a special (Wilson) flask.

‡ Percentage of essential oil found divided by a suitable factor. Factors are as follows: Orange, 0.95; lemon, 0.90; distilled lime, 0.79; expressed lime, 0.77; nutmeg, 0.75; peppermint, 0.61.

¶ Based on quantity taken.

CONCLUSIONS.

It is apparent from the summary of the data in Table 1 that the steam distillation method gives acceptable results in the case of oils of orange, lemon, and limes dissolved in corn and cottonseed oils, and that the results obtained on nutmeg and peppermint oils show that the application of the method to the determination of these essential oils is worthy of further consideration.

Determination of Anthranilic Acid Ester.

The methyl ester of anthranilic acid is universally employed to fortify grape concentrates and other food flavors that have a grape-like character. Many varieties of grapes contain anthranilic acid ester¹. The accurate determination of this ester is of such importance from a regulatory standpoint that after consultation with other interested persons, the referee considered it advisable to conduct collaborative tests on a colorimetric and a gravimetric method for its determination. Samples of known composition containing varying proportions of methyl anthranilate were prepared and submitted to 12 analysts, 11 of whom submitted their results.

The methods employed have been published^{1 2}. The results are given in Table 2.

PRECAUTIONS REGARDING THE COLORIMETRIC METHOD.

In diazotizing 88 mg. of methyl anthranilate 2 cc. of 2 per cent sodium nitrite solution will be used. Enough hydrazine sulfate to destroy completely the excess of nitrous acid present must be added; otherwise sodium nitrosonaphtholsulfonate, which is intensely yellow and modifies the color of the azo dye, is formed. The excess of this reagent must be kept as small as possible, however, in order to limit the number of side reactions.

The proper volume of the solutions of sodium-1-naphthol-2-sulfonate and of sodium carbonate should be measured in graduated cylinders when beginning the test. This procedure will enable the operator to add the reagents quickly, while keeping the solution in rotation, in such a way that each reagent will become incorporated in the liquid almost immediately. About 5 cc. of 25 per cent sodium carbonate solution is necessary to neutralize the hydrochloric acid present, but the addition of 15 cc. of this solution gives a solution containing equal parts of sodium carbonate and sodium bicarbonate, which Mathewson³ found to have the proper hydrogen-ion concentration to give a maximum color for this dye. The red dye formed by methyl anthranilate gradually saponifies

¹ *J. Agr. Research*, 1926, 33: 301.

² *This Journal*, 1928, 11: 46; *Ind. Eng. Chem.*, 1923, 15: 732.

³ *This Journal*, 1922, 6: 16.

in alkaline solution, forming the corresponding compound of anthranilic acid, which is a deeper yellow. For this reason it is necessary to apply the test reaction to standard solutions and distillates at as nearly the same time as possible.

TABLE 2.

Results of determination of anthranilic acid ester (methyl anthranilate).

COLLABORATOR*	METHOD NO. 1 COLORIMETRIC mg. per liter	METHOD NO. 2 GRAVIMETRIC grams per liter
L. W. Ferris	313	3.51
Buffalo, N. Y.	318	3.69
		3.91
		3.70
H. W. Haynes	328	3.82
Boston, Mass.	338	4.05
C. H. Hickey	328	3.50
Boston, Mass.	328	3.45
L. Jones	313	3.77
Chicago, Ill.	316	3.93
Walter Kirby	300	3.99
New York, N. Y.		
W. J. McCarthy	303	3.50
Cincinnati, O.	298	3.73
R. S. Roe	294	3.76
Chicago, Ill.	289	3.98
H. R. Smith	290	3.68
Baltimore, Md.	280	3.73
		3.70
W. C. Taber	323	1.66†
San Francisco, Calif.	329	1.66†
E. L. P. Treuthardt	274	3.83
Boston, Mass.	329	3.79
J. B. Wilson	316	3.65
Washington, D. C.		
Maximum	338	4.05
Minimum	274	3.45
Average	310	3.74
Present	313	3.92

* All collaborators are members of the U. S. Food, Drug and Insecticide Administration.

† Omitted from summary.

COMMENTS OF COLLABORATORS.

W. J. McCarthy.—Your specifications called for sealing the end of the condenser with a glass tube in order to make water seal in the Erlenmeyer flask. I fastened a thistle tube to the end of the condenser by means of a rubber stopper coated with tin foil.

It is suggested that some thought be given to substituting a standard flask for the abbreviated Kjeldahl flask, as specified in the method. I cut down an 800 cc. Kjeldahl flask to 10 inches over all, a 750 cc. flask not being available, but the cut-off flask was not easily fitted with a rubber stopper, either a No. 6 or a No. 7 size. On my original determinations it is suspected that the uniform low results were caused by a loss of the ester in the distillation.

H. R. Smith.—In general, weighing a precipitate on tared filter papers is not a satisfactory procedure. Accordingly the following method of filtration and weighing was tried: in addition to the duplicate determinations with the filter papers, a third portion of the distillate was treated as usual, but the precipitate was collected, dried, and weighed on a special Gooch crucible. This crucible contained a very thin pad of asbestos and about 15 grams of crystalline alundum, 90 mesh. Filtration was easy, and washing was more satisfactory than when filter papers were used. (The crystalline alundum is a new inert mechanical medium that promises to have many uses in the laboratory. It has been used for butter analysis and the determination of starch and crude fiber with marked success.)

CONCLUSIONS.

In considering the data in Table 2 it should be borne in mind that both of the methods are of such a character that experience with them is necessary to obtain acceptable results. The lack of such experience on the part of some of the collaborators is no doubt the cause of the rather wide deviations from the average. In general, the writer is of the opinion that the results are satisfactory and that the association will be warranted in adopting both of these methods as official.

RECOMMENDATIONS¹.

It is recommended—

(1) That the steam distillation method for the determination of oils of lemon, orange, and limes in corn and cottonseed oils and in mineral oil described in this report be adopted as official (first action).

(2) That the study of this method be continued with a view to extending its use to other non-alcoholic flavors.

(3) That the colorimetric method for the determination of small quantities of anthranilic acid ester described in this report be adopted as official (first action).

(4) That the gravimetric method for the determination of large quantities of anthranilic acid ester described in this report be adopted as official (first action).

REPORT ON MEATS AND MEAT PRODUCTS.

By R. H. KERR (Bureau of Animal Industry, Washington, D. C.),
Referee.

The work of the year was devoted entirely to the determination of total nitrogen in meats. No collaborative work was attempted owing to lack of collaborators. The work accomplished was carried out exclusively in the Washington and San Francisco Meat Inspection Laboratories of the Bureau of Animal Industry.

As pointed out in last year's report, the present official method for the determination of total nitrogen apparently calls for a longer period of digestion than is consistent with accuracy, particularly when modern

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 82.

electric heaters are used. There follow the results of a number of determinations of total nitrogen, or rather protein, since all results were calculated to protein by the formula $N \times 6.25$. These tests were made on approximately uniform samples of meat with varying proportions of reagents and methods of applying heat by H. R. McMillin, Bureau of Animal Industry, Washington, D. C., with one exception: A. E. Graham of San Francisco conducted Test XI.

TEST I.—Charge: 2–2.5 grams of imported frankfurter style sausage, total water content 54.2 per cent; 25 cc. of concentrated sulfuric acid; 15 grams of potassium sulfate (Kjelgest); and 1 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION hours	PROTEIN ($N \times 6.25$)
Gilmer Electric Heater	4	7.03 ¹
" " "	4	0.15 ²
Bunsen Burner	4	9.9
" " "	4	10.13
Gilmer Electric Heater	3	0.42 ¹
" " "	3	7.50 ¹
Bunsen Burner	3	10.37
" " "	3	9.4
Gilmer Electric Heater	2	8.76
" " "	2	9.25
Bunsen Burner	2	10.53
" " "	2	10.52
Gilmer Electric Heater	1	10.53
" " "	1	9.55 ³
" " "	1	10.72
" " "	1	10.55
" " "	1	10.59
Bunsen Burner	1	10.68
" " "	1	10.53

¹ Digestion stopped before end of 4 hour period on account of reduced volume of reacting mixture.

² Digestion stopped before end of 4 hour period on account of reduced volume of reacting mixture and softening of flask.

³ Omitted from average.

TEST II.—Charge: 2–2½ grams of sausage used in TEST I, 40 cc. of concentrated sulfuric acid, 15 grams of potassium sulfate (Kjelgest), and 1 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION hours	PROTEIN ($N \times 6.25$)
Gilmer Electric Heater	1	10.58
used throughout the	1	10.42
test	1	10.78
	1	10.75
	1	10.64
	2	10.62
	2	10.59
	2	10.63
	2	10.85
	2	10.59
	2	10.78
	3	10.53
	3	10.65
	3	10.71

TEST III.—Charge: 2.31–2.74 grams of domestic frankfurter style sausage, water content 65.0 per cent; 25 cc. of concentrated sulfuric acid; 15 grams of potassium sulfate (Kjelgest); and 1 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION	PROTEIN (N \times 6.25)
	<i>hours</i>	
Gilmer Electric Heater	1	17.70
" " "	1	17.82
" " "	1	17.87
Average		17.79
Bunsen Burner	4	17.60
" "	4	17.81
" "	4	17.56
Average		17.66

TEST IV.—Charge: 2.31–2.74 grams of sausage used in TEST III, 40 cc. of concentrated sulfuric acid, 15 grams of potassium sulfate (Kjelgest), and 1 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION	PROTEIN (N \times 6.25)
	<i>hours</i>	
Gilmer Electric Heater	2	17.78
used throughout the	2	17.64
test	2	17.80
Average		17.74

TEST V.—Charge: 2–2½ grams of fresh pork sausage, water content 40.2 per cent; 25 cc. of concentrated sulfuric acid; 15 grams of potassium sulfate (Kjelgest); and 1 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION	PROTEIN (N \times 6.25)
	<i>hours</i>	
Gilmer Electric Heater	1	3.42
" " "	1	0.10
" " "	1	0.10
" " "	1	0.12
Bunsen Burner	4	9.4
" "	4	0.37
" "	4	10.65
" "	4	6.8

TEST VI.—Charge: 2–2½ grams of sausage used in TEST V, 40 cc. of concentrated sulfuric acid, 15 grams of potassium sulfate (Kjelgest), and 1 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION	PROTEIN (N \times 6.25)
	<i>hours</i>	
Gilmer Electric Heater	2	10.74
used throughout the	2	10.68
test	2	10.88
	2	10.77
With asbestos guard	3½	10.94

TEST VII.—Charge: 2-2½ grams of sausage used in TESTS V and VI, 25 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate (Kjelgest), and 0.3 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N × 6.25)
Gilmer Electric Heater	1	10.88
“ “ “	1	10.80
“ “ “	1	10.87
Average		10.85
Bunsen Burner	4	10.82
“ “	4	10.96
“ “	4	10.91
Average		10.89

TEST VIII.—Charge: 2-2½ grams of sausage used in TESTS V, VI, and VII; 40 cc. of concentrated sulfuric acid; 10 grams of potassium sulfate (Kjelgest); and 0.3 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N × 6.25)
Gilmer Electric Heater	2	10.82
used throughout the	2	10.87
test	2	10.82
Average		10.84

TEST IX.—Charge: 2-2½ grams of frankfurter style sausage, water content 59.0 per cent; 25 cc. of concentrated sulfuric acid; 10 grams of potassium sulfate (Kjelgest); and 0.3 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N × 6.25)
Gilmer Electric Heater	1	16.90
used throughout the	1	17.00
test	1	16.96
Average		16.95

TEST X.—Charge: 2-2½ grams of sausage used in TEST IX, 40 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate (Kjelgest), and 0.3 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N × 6.25)
Gilmer Electric Heater	3½	17.32
with asbestos guard	3½	17.48
used throughout the	3½	17.50
test		
Average		17.43

TEST XI.—Charge: 2-2½ grams of sausage used in TESTS IX and X, 50 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate, and 2 cc. of saturated solution of copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N \times 6.25)
Bunsen Burner used throughout the test	2	16.53
	4	17.04
	6	17.06
	8	17.29

TEST XII.—Charge: 2-2½ grams of pork sausage, water content 47.8 per cent; 25 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate (Kjelgest), 0.3 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N \times 6.25)
Gilmer Electric Heater used throughout the test	40	14.76
	40	14.85
	40	14.65
		<hr/>
	Average	14.75
	60	14.86
	60	14.82
	60	14.80
		<hr/>
	Average	14.83

TEST XIII.—Charge: Same as TEST XII except that 40 cc. of concentrated sulfuric acid was used instead of 25 cc.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N \times 6.25)
Gilmer Electric Heater used throughout the test	1	14.55
	1	14.38
	1	14.45
		<hr/>
	Average	14.46
	2	14.76
	2	14.81
	2	14.75
		<hr/>
	Average	14.77

TEST XIV.—Charge: Same as TEST XII except that 50 cc. of concentrated sulfuric acid was used instead of 25 cc.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N \times 6.25)
Gilmer Electric Heater used throughout the test	1	14.44
	1	14.35
	1	14.28
		<hr/>
	Average	14.36
	2	14.82
	2	14.72
	2	14.64
		<hr/>
	Average	14.73

TEST XV.—Charge: 0.5 gram of dried horse meat, water content 5.14 per cent; 25 cc. of concentrated sulfuric acid; 10 grams of potassium sulfate (Kjelgest); and 0.3 gram of crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N \times 6.25)
Gilmer Electric Heater	1	66.32
used throughout the	1	66.32
test	1	66.34
		<hr/>
		Average 66.32

TEST XVI.—Charge: 0.5 gram of horse meat used in TEST XV, 40 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate (Kjelgest), and 0.3 gram crystallized copper sulfate.

SOURCE OF HEAT	TIME OF DIGESTION <i>hours</i>	PROTEIN (N \times 6.25)
Gilmer Electric Heater	4	67.02
with asbestos guard	4	66.94
used throughout the	4	67.1
test		<hr/>
		Average 67.02

The results given in these numerous tests show that digestion for such periods of time as specified in the official method is impractical, particularly when electric heaters are used. Accordingly, recommendation is being made for an appropriate change in the method.

In this connection attention is called to the fact that the highest results reported are those in which an asbestos guard was used in connection with the electric heater. This guard consisted of a thin asbestos plate with a circular opening $2\frac{5}{8}$ inches in diameter laid flat on top of the heater. The effect was to prevent contact of the flask with the curved inner surface of the bowl-like depression in the upper brick of the heater and to leave the flask approximately one inch above the coils. Contact of the flask with the curved inner surface of the upper brick of the heating apparatus results in overheating of the sides of the flask above the surface of the reaction mixture, thereby bringing about loss of nitrogen. This is proved by the fact that results obtained when the guard is used are uniformly higher than those obtained without it, all other conditions being the same. Accordingly, the use of an asbestos guard with this type of heater is recommended. It is also the intention of the referee to bring this source of error to the attention of the manufacturers of the apparatus.

RECOMMENDATION¹.

It is recommended that the words, "In the Kjeldahl and Gunning methods digest with sulfuric acid for at least 4 hours; in the Kjeldahl-

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 82.

Gunning-Arnold Method, for 2 hours after the mixture has become clear", be deleted from the official method for total nitrogen in meats, *Methods of Analysis, A. O. A. C.*, 1925, p. 237. The method will then read as follows: "Proceed as directed on p. 7, 19, or p. 8, 22, or 24, using about 2 grams of the fresh sample". The effect of this change will be to leave the directions for digestion the same as in Chapter I, 19, 22, or 24.

No report on the separation of meat proteins was given by the associate referee.

No report on gelatin was given by the referee.

No report on spices and other condiments was given by the referee.

REPORT ON CACAO PRODUCTS.

By H. A. LEPPER (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

No work was done by the referee this year. A report on crude fiber in cacao products was presented by Marie L. Offutt, who substituted for the associate referee appointed, E. R. Miller, and W. F. Baughman presented a report on the detection of coconut oil and palm kernel oil in cacao butter and fat from milk chocolate.

In keeping with the recommendations of Offutt, it is recommended¹—

(1) That the method proposed for crude fiber be studied further, and that collaborative work be done.

(2) That modifications of the method applicable to milk cacao products be studied.

In keeping with the recommendations of Baughman, it is recommended—

(1) That the method as described in the report of the associate referee for the detection of coconut oil and palm kernel oil in cacao butter and in fat from milk chocolate be adopted as a tentative method.

(2) That study be continued on methods for the detection of foreign fats in cacao fat and fat from cacao products in general.

It is noted that the method for the detection of cacao shell given in *Methods of Analysis, A. O. A. C.*, 1925, p. 347, is not designated as a

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 83.

tentative method. This method was adopted tentatively at the 1921 meeting¹.

It is therefore recommended that the method be designated as "tentative", and also that the method for coloring matters on the same page be so designated.

It is further recommended—

(1) That the official method for the determination of fat in cacao products be dropped (final action).

(2) That methods for the determination of milk solids and sucrose be studied.

No report on microscopic methods for cacao products was given by the associate referee.

REPORT ON CRUDE FIBER IN CACAO PRODUCTS.

By MARIE L. OFFUTT² (U. S. Food, Drug and Insecticide Administration, New York, N. Y.), *Associate Referee*.

The present method for determining crude fiber in cacao products³ is somewhat defective, for the reason that the quantity of sample used varies according to the amount of sugar and other substances added during manufacture. Varying quantities of sugar and water-soluble cacao material are present in the acid solution, and they may influence the solubility of the other constituents of the sample in the reagent. Furthermore, it may be necessary to use a large quantity of material to obtain a sample sufficiently large to yield the 1 gram of cacao material required, and difficulties are often encountered in filtration. In addition, a factor—which at times may be quite large—has to be used to convert the crude fiber, as determined, to the basis on which it is to be reported.

A modification of this method, developed in the New York Station of the U. S. Food, Drug and Insecticide Administration, provides for operating on a 2 gram sample consisting of the ether, water- and alcohol-insoluble material. The object in modifying the method was to find some constant in cacao material which is present to a great extent in the shell, and also, to a much lesser extent, in the nib material, in order to determine the adulteration of cacao products with added cacao shell. Determinations of material insoluble in various reagents, such as dilute acids and alkalis of varying strengths and combinations of these, were attempted, but the extent of variation between shell and nib material was not found to be so great as had been expected.

¹ *This Journal*, 1922, 6: 150.

² Substituted during the year for the associate referee appointed, E. R. Miller.

³ *Methods of Analysis*, A. O. A. C., 1925, 347.

The next step in the investigation involved the determination of crude fiber on residues obtained from treatment of the cacao material with various solvents by using the insoluble residue remaining after extraction of the material with hydrochloric acid—0.1-5 *N*—varying strengths of ammonia, and ether, alcohol, and water. It was found that the greatest variance resulted from the use of ether, alcohol, and water. The method as finally developed was as follows:

Treat 15 grams of liquor or 50 grams of sweet chocolate in a nursing bottle with 100 cc. of ether, centrifuge, and decant the supernatant liquor twice; dry the residue in an oven at about 100°C. and then powder in the bottle with a flattened glass rod. (In some cases it may be necessary to grind the material in a mortar and extract a third time with ether.) Wash in the nursing bottle with three 100 cc. portions of distilled water at room temperature, shaking well each time until no cacao material adheres to the bottle. Centrifuge after each washing for 10-15 minutes and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc. portions of 95 per cent alcohol and one 100 cc. portion of ethyl ether. Transfer the residue to a platinum dish, dry to constant weight, and grind in a mortar. Weigh 2 grams of the dried material and determine crude fiber by the usual A. O. A. C. method¹. Report results as the percentage of crude fiber in the washed and dried material.

Eight samples were examined by this method in comparison with the tentative A. O. A. C. method with the following results:

SAMPLE NO.	CRUDE FIBER TENTATIVE METHOD	CRUDE FIBER DETERMINED ON ETHER, ALCOHOL- AND WATER-INSOLUBLE MATERIAL		
		1st Run	2nd Run	3rd Run
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
83	7.25	8.60 8.51		
84	6.54	7.99 8.12		
83	7.18	9.14 9.26	9.22 9.29	9.12 9.18 9.14
84	6.31	8.18 8.00		
30	6.56	8.70 8.60		
70	6.60	8.69 8.84		
306	8.32	8.27 8.17		
53	15.30	10.15 10.07		

Determinations were also made on a series of roasted nibs free from shell and on shell material free from nibs. The following results were obtained:

¹ *Methods of Analysis*, A. O. A. C., 1925, p. 117.

SAMPLE NO.	MATERIAL	SHELL	CRUDE FIBER
		per cent	per cent
418	Arriba Nibs	0	8.15
419	Bahia Nibs	0	7.81
420	Caracas Nibs	0	7.85
423	Accra Nibs	0	8.50
424	Bahia Nibs	0	8.82
425	Sanchez Nibs	0	7.84
776	Mixed Nibs	0	8.30
781	Accra Nibs	0	7.94
783	Machala Nibs	0	8.93
784	Sanchez Nibs	0	7.59
785	San Felipe Nibs	0	8.33
786	Trinidad Nibs	0	7.97
792	Accra Nibs	0	8.09
793	Bahia Nibs	0	8.34
794	Caracas Nibs	0	8.39
777	{ Accra Shell	100	25.46
	{ Sanchez Shell		
778	{ Sanchez Shell	100	23.40
779	{ Trinidad Shell	100	25.92
	{ Arriba Shell	100	26.25
787	{ Bahia Shell		
789	{ Accra Shell	100	25.41
790	{ Bahia Shell	100	26.67
791	{ Caracas Shell	100	22.94
797	{ Arriba Shell	100	20.61
800	{ Lagos Shell	100	24.11
801	{ Pt. Cabella Shell	100	20.06

The results obtained by the modified method seem to show greater variations for shell material and nib material than those obtained by the usual method for crude fiber in cacao products. The method, however, is not applicable in its present form to milk chocolates, and further work must be done to make it useful for that class of goods. However, it does seem to be applicable to cocoa, bitter liquors, and sweet chocolates.

RECOMMENDATIONS.¹

It is recommended—

(1) That the method given be further studied and later adopted as a tentative method for the determination of crude fiber in cocoa, bitter liquors, and sweet chocolates.

(2) That during the coming year the associate referee study modifications of the method with a view to making it applicable to cacao products containing milk solids.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 84.

REPORT ON CACAO BUTTER.

By WALTER F. BAUGHMAN (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*.

This report relates to the work done in formulating a qualitative test for the detection of adulteration of cacao butter and milk chocolate with coconut oil and palm kernel oil. The test was suggested by Cohn¹ and Strube². It has been published³.

The part of the test outlined under the heading "Examination of Cacao Butter" was submitted to six collaborators in 1924. They applied it to six samples, the composition of which was unknown to them. Four of the samples consisted of cacao butter, milk fat, and either coconut oil or palm kernel oil. One sample was a mixture of cacao butter and milk fat, and the other sample was pure cacao butter. All six collaborators reported positive results for the first four samples and negative results for the sixth sample. For the fifth sample, consisting of cacao butter and milk fat, four collaborators reported "very faintly positive" results, one a "faintly positive" result, and the other one a positive result⁴. These results show that the test is satisfactory for detecting coconut oil and palm kernel oil in cacao butter, but that as then described it could not be used when milk fat is present.

This year an effort was made to modify the test in order to make it satisfactory for examining fat extracted from milk chocolate, chocolate fondant, covering chocolate, etc. Milk fat, as well as coconut oil and palm kernel oil, contains caproic, caprylic, and capric acids as glycerides although in smaller quantities, and these are the acids that cause the turbidity when the last filtrate is acidified. They can not be separated entirely by extending the fractional salting out of the soaps; even the last filtrate from the sample containing milk fat becomes slightly turbid upon acidifying. However it is believed that the test can be made satisfactory for milk chocolate fat by using as a blank a mixture of cacao butter and milk fat in the proportions in which they occur in the fat separated from the milk chocolate product under examination.

The percentage of lactose and casein in the product, either one or both, and of total fat will have been determined in the course of analysis. The percentage of lactose, multiplied by the factor 0.8, or the percentage of casein, multiplied by the factor 1.1, gives the percentage of milk fat in the product. From this figure and the percentage of total fat in the product, is calculated the percentage of milk fat in the total fat. The analyst then has the necessary information for making up the mixture

¹ *Chem. Ztg.*, 1907, 31: 855.

² *Z. offent. Chem.*, 1908, 14: 67.

³ *This Journal*, 1925, 8: 703; 1928, 11: 45.

⁴ *Ibid.*, 1925, 8: 704.

of cacao butter and milk fat to be used as the blank. These lactose and casein factors have been calculated from the ratios of lactose to fat (36.5 : 28) and casein to fat (26 : 28) in standard whole milk powder, and of course they hold good for concentrated whole milk and whole milk.

Milk chocolate product usually does not contain more than 8 per cent milk fat, and the average figure for total fat is 30–35 per cent. An error of 2 per cent in the calculated figure for milk fat in the product would increase to an error of 5–7 per cent for milk fat in the total fat. Such an error would be of no consequence to the analyst because this quantity of milk fat in cacao butter does not cause an opalescence in the final filtrate when acidified. Therefore no great accuracy is necessary in this calculated percentage of milk fat. In the literature¹ are published the results of many analyses of milk chocolates, and the determined figures for lactose, casein, milk fat, and fat are given. When the percentages of milk fat are calculated from the figures for lactose and casein by means of the factors given in the description of the test, with few exceptions the calculated percentages check closely with the determined figures.

Seven "unknown" samples, the compositions of which are given in Table 1, and Sample A, which was a mixture of 80 per cent cacao butter and 20 per cent milk fat, were sent to each collaborator with a request to apply the test specified to the unknown samples and to use Sample A as a blank.

The results reported by the collaborators, members of the U. S. Food, Drug and Insecticide Administration, are given in Table 1. All six analysts reported positive results for Samples 1, 2, 4 and 5, which consisted of cacao butter, milk fat and either coconut oil or palm kernel oil in various proportions. For Sample 3, which was composed of cacao butter and milk fat in the same proportions as the blank, five analysts reported negative results and the other analyst reported a "very faintly positive" result. Four collaborators reported negative results and two reported "faintly positive" results for Sample 6, which contained no coconut oil or palm kernel oil and 5 per cent more milk fat than the blank. Sample 7 contained no coconut oil or palm kernel oil and 10 per cent more milk fat than the blank and for this sample there were obtained two negative results, three "faintly positive results" and one positive result.

The results for Samples 6 and 7 indicate that the analyst has some leeway in calculating the percentage composition of the blank, and if he should get the blank made up with 5–6 per cent less milk fat than the sample, it would not cause him to report, as adulterated, the fat from a pure milk chocolate product.

¹ Whymper. *Cocoa and Chocolate*, 2nd ed., p. 290; Booth, Cribb and Richards. *Analyst*, 1909, 34: 146.

TABLE 1.
Results of collaborative work.

ANALYST	SAMPLE 1 (72% cacao butter 18% milk fat 10% coconut oil)	SAMPLE 2 (64% cacao butter 16% milk fat 20% coconut oil)	SAMPLE 3 (80% cacao butter 20% milk fat)	SAMPLE 4 (72% cacao butter 18% milk fat 10% palm kernel oil)	SAMPLE 5 (64% butterfat 10% milk fat 20% palm kernel oil)	SAMPLE 6 (75% cacao butter 25% milk fat)	SAMPLE 7 (70% cacao butter 30% milk fat)
H. R. Smith Baltimore, Md.	positive	strongly positive	negative	faintly positive	faintly positive	negative	faintly positive
M. L. Offutt New York, N. Y.	"	positive	"	positive	positive	"	negative
Herman W. Haynes Boston, Mass.	"	"	"	"	"	"	positive
J. Fitelson Philadelphia, Pa.	"	"	"	faintly positive	"	faintly positive	faintly positive
Llewelyn Jones Chicago, Ill.	"	"	very faintly positive	positive	"	"	"
J. I. Palmore Washington, D. C.	strongly positive	strongly positive	negative	faintly positive	"	negative	negative

After comparing the results for this year with the results obtained in 1924, it is concluded that the test can now be used satisfactorily for the detection of coconut oil and palm kernel oil in cacao butter or the fat from milk chocolate.

RECOMMENDATION¹.

It is recommended that the method formulated by the associate referee for the detection of coconut oil and palm kernel oil in cacao butter and fat from milk chocolate be adopted as a tentative method.

No report on naval stores was given by the referee.

No report on turpentine was given by the associate referee.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1928, 11: 84.

CONTRIBUTED PAPERS.

FORMULA FOR CALCULATING COMPOSITION OF MIXTURES OF MYDRIATIC ALKALOIDS.

By J. C. MUNCH and G. S. GITTINGER (Food, Drug and Insecticide Administration, Washington, D. C.).

In connection with another investigation conducted by this Administration, the necessity arose for a method of determining quantitatively the composition of solutions containing various mydriatics. In some instances, as in the study of the change of hyoscyamine to atropine, it was believed that the test solutions contained a mixture of both alkaloids.

Tests were conducted by the "cat-eye" method developed by J. C. Munch and reported at the meeting of the Association of Official Agricultural Chemists in 1926.¹ Based upon results of chemical assay, various dilutions were tested in order to determine the particular dilution that would cause perceptible mydriasis, as outlined in the description of the method. A formula was desired whereby it would be possible to determine the quantities of each of the constituents in a solution containing two mydriatics. The more interesting and difficult problem of computing the relative quantities of three mydriatic alkaloids when present in the same solution has not been undertaken because this step would necessitate an independent determination as well as the physiological assay.

A number of solutions containing atropine mixed with hyoscyamine, cocaine, or homatropine were tested. In every instance it was found that the resultant physiological effect of the solutions was a direct summation of the physiological actions of the two constituents. No evidence of potentiation was found with any of these mixtures. However, it is interesting to note that cocaine and ephedrine hydrochloride or ephedrine sulfate apparently potentiate their action by some 25 per cent.

In the example to be discussed here, atropine and hyoscyamine solutions will be used. Experiments showed that 12 mg. of atropine per liter gave a solution of which one drop was just capable of causing perceptible mydriasis in the cat's eye. Similarly, 4 mg. of hyoscyamine per liter produced the same physiological action. Solutions containing respectively 9 mg. of atropine and 1 mg. of hyoscyamine, 6 mg. of atropine and 2 mg. of hyoscyamine, and 3 mg. of atropine and 3 mg. of hyoscyamine were found to have exactly the same physiological activity as the indicated concentrations of the single alkaloids. Computing the values for total alkaloids, which means the sum of the atropine and of the hyoscyamine

¹ *This Journal*, 1927, 10: 383.

when expressed as milligrams per liter, as abscissae, and computing the values for atropine as ordinates, the results obtained with mixtures of the solutions in varying proportions fell on a straight line.

It is self-evident that if atropine alone is present in a solution the total alkaloids will be only atropine and the threshold concentration will contain 12 mg. of total alkaloid per liter. If only hyoscyamine is present, the total alkaloids will constitute 4 mg. per liter. Mixtures of these two will give intermediate results. It follows, then, that in making a series of solutions ranging in composition from no atropine plus 4 mg. of hyoscyamine to a solution containing 12 mg. of atropine plus no hyoscyamine, the increase of 12 mg. in atropine content correlative with a decrease of 4 mg. in hyoscyamine content produces a net increase of 8 mg. in total alkaloids. Since 3 mg. of atropine is physiologically equal to 1 mg. of hyoscyamine, there is no change in the physiological activity throughout this entire series of solutions. Since an increase of 12 mg. of atropine produces an increase of 8 mg. in total alkaloids, 1.5 mg. of atropine will give an increase of 1 mg. of total alkaloids. Increase in alkaloid content commences with a solution that contains 4 mg. of total alkaloid (hyoscyamine). Accordingly, if X represents the total alkaloid content, $X - 4$ will represent the increase in alkaloid content. Then if Y represents the atropine content of a solution containing atropine and hyoscyamine, the equation expressing the relation between Y (atropine content) and X (total alkaloid content) becomes $Y = 1.5 (X - 4)$, which simplifies to $Y = 1.5 X - 6$. That is to say, the atropine content of a solution containing atropine and hyoscyamine is 6 mg. less than $1\frac{1}{2}$ times the total alkaloidal content.

This example has been worked out in detail in order to show the general derivation of the formula for computing the constituents of such mixtures. Considering the several portions of this equation in detail, a general formula is readily deduced. The value 1.5 is obtained by dividing 12 by 8; 12 is the threshold concentration of atropine; 8 is obtained by subtracting the threshold concentration of hyoscyamine, which is 4, from the threshold concentration of atropine, which is 12. Accordingly, the value for the coefficient of the expression in parenthesis is the result obtained by dividing the threshold of one mydriatic by the difference in thresholds between the two mydriatics known to be present in the mixed solution. In more mathematical terminology, $Y = M (X - A)$ is the equation of a straight line fitting the results in question, and the value just obtained is the value for M . To avoid negative results, it is advisable to substitute for the threshold in the numerator the value that is numerically the greatest. The two mydriatics under consideration may then be considered as A and B , and the equation becomes

Threshold A
Threshold A — Threshold B

The expression in parenthesis is then Total Alkaloid minus Threshold *B*. In the example used, *A* was taken to be atropine with a threshold of 12 and *B*, hyoscyamine with a threshold of 4. The value for *Y* is then the value for mydriatic *A* in terms of milligram per liter. It is obvious that if but two alkaloids are present and the quantity of one alkaloid has been determined, the remainder of the total alkaloid consists of the other product. Accordingly, the method of determining the quantity of one mydriatic in a solution containing two mydriatics is as follows:

(1) By the preparation of various dilutions, determine the concentration in milligrams per liter that is capable of causing satisfactory mydriasis.

(2) Substitute the proper values in the following equation:

$$\text{Concentration Mydriatic } A = \frac{\text{Threshold } A}{\text{Threshold } A - \text{Threshold } B} (\text{Total Alkaloid} - \text{Threshold } B).$$

This formula has been successfully applied to mixtures of various mydriatics without difficulty.

THE IODINE NUMBER OF SPANISH PAPRIKA OIL.

By LLOYD C. MITCHELL and SAMUEL ALFEND (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.)¹.

The analyses of authentic samples of paprika reported by Tolman and Mitchell² were used in judging the purity of ground Spanish paprika, particularly in detecting added olive oil. The modified Doolittle-Ogden method³ for the extraction of the oil and the determination of its iodine number has been practically superseded by the chloroform extraction method⁴. Considerable work by the writers⁵ in this laboratory, however, resulted in a slight modification of the latter method that gave iodine values 3-4 units higher than those obtained by the original method.

The limits for the iodine number of paprika oil given in the Standards⁶ are 125-136, but samples of paprika imported into this country in recent years have consistently shown iodine numbers over 130, and usually over 135, by the method now in use. Mitchell⁷ has reported the analyses of five authentic samples of Spanish paprika that were prepared from the whole pods of the 1926 crop. The amount of extracted oil ranged from

¹ Ernest R. Smith, Chief of Station.

² U. S. Dept. Agr. Bur. Chem. Bull. 163.

³ *J. Am. Chem. Soc.*, 1908, 30: 1481.

⁴ *This Journal*, 1926, 9: 477.

⁵ Unpublished.

⁶ U. S. Dept. Agr., Food, Drug and Insecticide Adm., S. R. A., F. D. No. 2, p. 13.

⁷ Unpublished.

10.33 to 11.01 per cent, and the iodine values ranged from 139 to 146. It seems evident, therefore, that the present standards, 125-136, cannot be used to judge iodine numbers determined by the chloroform extraction method.

With a view to determining the iodine number of the oil in authentic samples of Spanish paprika, Mitchell obtained through the courtesy of Eme Flores, of the firm of Francisco Flores, Espinardo, Murcia, Spain, samples of pods grown in eleven districts in 1927. These samples were received December 1, 1927. The pods in each lot were strung together by a cord through the stems. During the month of December they were separated into shells, seeds, placentae, and stems. The various divisions were placed in air-tight containers and kept in a dark place until May 31, 1928, when they were ground. The separated portions were later re-assembled in their original proportions (Series A samples) and in other proportions (Series B and C samples), and analyzed June 1 and 2, 1928. The stems were not included in the samples. The Series B samples were prepared with 70 per cent shells and 30 per cent seeds and placentae, the natural ratio for seeds and placentae being maintained for each sample. The Series C samples were prepared with 45 per cent shells and 55 per cent seeds and placentae.

It is understood that it is the commercial practice to separate the shells from the seeds and placentae, dry them separately, and then combine them before grinding in proportions varying according to the grade or quality sought. It is believed that the proportions used in this work cover the range of proportions found in the commercial product.

Many of the pods had lost much of their red color, and the chloroform extracts were not so deep red as the extracts obtained from commercial samples. However, aging experiments just completed in this laboratory show that the iodine number of the oil is not affected by loss of color in the shell or in the chloroform extract.

EXPERIMENTAL WORK.

The method used is as follows:

Transfer 10 grams of the ground sample to a 200 cc. glass-stoppered flask and add 100 cc. of chloroform from a pipet, rotating while adding the first 50 cc. Let stand 1 hour, shake, and filter through a 12.5 cm. fluted filter. Pipet off successively two 10 cc. portions, using the same pipet. Transfer one of the 10 cc. portions to a weighed crystallizing dish, 50 x 35 mm., and evaporate the solvent by placing the dish on a steam bath. Dry the dish and contents at 100°C. for 1 hour, cool in air, and weigh. Use the weight obtained in calculating the iodine number. Transfer the other 10 cc. portion to a suitable glass-stoppered flask or bottle for the determination of the iodine number. Add 30 cc. of Hanus solution and follow the official method (*Methods of Analysis*, A. O. A. C., 1925, p. 287).

The results of the separations, with comments on the condition of the samples, are given in Table 1. The proportions in which the samples

were made up are given in Table 2. The results of analyses are given in Tables 3, 4, and 5.

TABLE 1.
Composition of the pods.

SAMPLE NUMBER	DISTRICT GROWN	NUMBER OF PODS	STEM-FREE BASIS			STEMS
			Shells	Seeds	Placentae	
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	Alhama	64	60.8	34.6	4.6	7.4
2	Aljorra	32	54.3	38.7	7.0	7.2
3*	Beniel	77	55.0	38.9	6.1	7.7
4	Calasparra	55	57.9	36.6	5.5	7.1
5†	Elche	33	54.9	36.3	8.8	7.4
6	Esparragal	38	56.7	38.9	4.4	6.2
7‡	Espinardo	32	54.7	37.3	8.0	6.1
8	La Ribera	22	56.7	37.4	5.9	5.8
9	Molina	33	60.2	35.1	4.7	7.4
10	Montesinos	43	57.5	38.5	4.0	6.9
11	Santomera	50	53.9	41.9	4.2	6.9

* About 50% moldy.

† Moist and shriveled.

‡ Some moldy; all badly shriveled.

TABLE 2.
Proportions of separations used for samples.

SAMPLE NUMBER	SERIES A			SERIES B			SERIES C		
	Shells	Seeds	Placentae	Shells	Seeds	Placentae	Shells	Seeds	Placentae
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	60.8	34.6	4.6	70.0	26.4	3.6	45.0	48.3	6.7
2	54.3	38.7	7.0	70.0	25.4	4.6	45.0	46.6	8.4
3	55.0	38.9	6.1	70.0	25.9	4.1	45.0	47.5	7.5
4	57.9	36.6	5.5	70.0	26.1	3.9	45.0	46.9	8.1
5	54.9	36.3	8.8	70.0	24.1	5.9	45.0	44.3	10.7
6	56.7	38.9	4.4	70.0	27.0	3.0	45.0	49.4	5.6
7	54.7	37.3	8.0	70.0	24.9	5.1	45.0	45.6	9.4
8	56.7	37.4	5.9	70.0	25.9	4.1	45.0	47.5	7.5
9	60.2	35.1	4.7	70.0	26.4	3.6	45.0	48.5	6.5
10	57.5	38.5	4.0	70.0	27.2	2.8	45.0	49.8	5.2
11	53.9	41.9	4.2	70.0	27.3	2.7	45.0	50.0	5.0

DISCUSSION.

The agreement in iodine numbers between the analogous samples in Series A and B is quite good, the maximum variation between analogous samples being 1.7 and the average variation 0.7. The averages for the eleven samples are the same, 136.5, in both series.

It is interesting to note that the samples in Series C, which contains the most oil, are uniformly lower in iodine number than the samples in Series A and B, the average value, 134.5, being two units lower than the average for the other two series.

TABLE 3.
Results of analyses—Series A samples.
(Shells, seeds, and placentae in natural ratio.)

SAMPLE NUMBER	CHLOROFORM EXTRACT			
	Total	Non-Volatile	Volatile	Iodine Number*
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1	10.52	10.37	0.15	136.6
2	10.44	10.29	0.15	138.9
3	11.16	10.98	0.18	137.0
4	10.71	10.53	0.18	134.8
5	11.38	11.14	0.24	134.0
6	10.74	10.55	0.19	137.5
7	10.74	10.54	0.20	134.7
8	9.53	9.32	0.21	137.8
9	9.69	9.53	0.16	136.9
10	11.37	11.21	0.16	137.4
11	11.60	11.39	0.21	135.8
Maximum	11.60	11.39	0.24	138.9
Minimum	9.53	9.32	0.15	134.0
Average	10.72	10.53	0.19	136.5

* To save time the method directs the evaporation and immediate heating of the chloroform extract; the weight used, therefore, is that of the non-volatile extract, whereas the iodine absorption is obtained on the total chloroform extract.

TABLE 4.
Results of analyses—Series B samples.
(Ratio: shells, 70 per cent; seeds and placentae, 30 per cent.)

SAMPLE NUMBER	CHLOROFORM EXTRACT			
	Total	Non-Volatile	Volatile	Iodine Number*
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1	9.36	9.20	0.16	136.5
2	8.61	8.47	0.14	139.7
3	9.63	9.51	0.12	136.0
4	8.74	8.60	0.14	134.9
5	9.60	9.46	0.14	133.6
6	9.26	9.05	0.21	138.8
7	9.18	9.09	0.09	134.0
8	8.06	7.94	0.12	138.4
9	8.65	8.47	0.18	135.2
10	9.83	9.59	0.24	138.2
11	8.74	8.48	0.26	135.9
Maximum	9.83	9.59	0.26	139.7
Minimum	8.06	7.94	0.09	133.6
Average	9.06	8.90	0.16	136.5

* See footnote, Table 3.

Sample 2 had the highest iodine number in Series A and B, and the next to the highest in Series C. Sample 5 had the lowest value in Series A and B, and the next to the lowest in Series C.

It is observed in Table 1 that Samples 5 and 7, which average about the lowest in all three series, were moldy and shriveled.

TABLE 5.

Results of analyses—Series C samples.

(Ratio: shells, 45 per cent; seeds and placentae, 55 per cent.)

SAMPLE NUMBER	CHLOROFORM EXTRACT			
	Total	Non-Volatile	Volatile	Iodine Number*
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	13.46	13.22	0.24	134.2
2	12.38	12.25	0.13	135.8
3	13.10	12.96	0.14	136.4
4	12.55	12.44	0.11	133.0
5	13.20	13.07	0.13	133.2
6	12.83	12.77	0.06	134.4
7	12.65	12.52	0.13	134.2
8	11.33	11.23	0.10	134.4
9	12.20	12.12	0.08	134.6
10	13.70	13.57	0.13	134.8
11	13.30	13.16	0.14	134.3
Maximum	13.70	13.57	0.24	136.4
Minimum	11.33	11.23	0.06	133.0
Average	12.79	12.66	0.13	134.5

* See footnote, Table 3.

SUMMARY AND CONCLUSIONS.

Analyses of eleven authentic samples of paprika grown in various districts in Spain in 1927 are submitted. The pods were broken up, and the shells, seeds, and placentae were combined in various proportions. The iodine numbers were determined by the chloroform extraction method given in detail in this report.

The iodine numbers obtained ranged from 133.0 to 139.7. The lowest iodine numbers were obtained in the series containing the largest proportion of seeds, and therefore of oil. The two samples having the lowest iodine numbers were moldy or shriveled, and therefore the results are open to suspicion.

The iodine number range specified by the official standards is not applicable to samples analyzed by the chloroform extraction method. The majority of the results reported for authentic samples are over the maximum limit, 136, and all of them are over 130.

BOOK REVIEWS.

Einheitliche Untersuchungsmethoden für die Fett-Industrie. First Part, edited and produced by the Wissenschaftlichen Zentralstelle für Öl und Fettforschung. E. V. BERLIN. Pp. XVI + 105. Stuttgart, 1927. Wissenschaftliche Verlagsgesellschaft m. b. h.

The compilers of this book have attempted to provide uniform methods of examination and to establish standard analytical procedures for those engaged in the analytical control of the raw materials and the products of the fat and soap industries, the object being to eliminate differences of standard between buyer and seller. The book contains detailed directions for obtaining average samples, tests for detecting impurity and adulteration of oils and fats, methods for determining their usual characteristics and methods for examining soaps, soap powders, and glycerine. The methods have been well selected, are clearly described, and a simple formula for calculating the results is provided for each quantitative method. Since only one method for each test and determination is given, the general scope of the book may be too limited for the larger laboratories, but it should be valuable to the laboratories of smaller works.—
WALTER F. BAUGHMAN.

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